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Genetic Association of MCP-1 *rs*1024611 Polymorphism with Susceptibility of Type 2 Diabetic Foot Ulcer from Punjab, Pakistan

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RB: Conceived the study, participated in the design of the study and performed the statistical analysis. SB, RB: Sample collection, obtained ethical clearance and permission for study. KL, SR and MA: Drafting the manuscript or revisiting it critically for important intellectual content.

Key words
***MCP-1, rs1024611*, Diabetic foot ulcer, T2DM, T allele, Pakistani population**

ABSTRACT

Diabetic foot ulcer (DFU) is a worldwide health problem and most common in underdeveloped countries. Monocyte Chemoattractant Proteins-1 (*MCP-1*) is a chemokines that plays important role in inflammatory response and attract monocytes. The purpose of this study was to investigate risk alleles of *rs1024611* polymorphism of *MCP-1* gene in Pakistani DFU patients. This case control study was comprised of total 105 type 2 diabetes patients with complication of foot ulcer and 117 patients without foot ulcer along with 100 healthy Control. All patients were clinically investigated by questionnaire directed through interviews. Genetic association study was performed by genotyped the *MCP-1* polymorphism (rs1024611) by Amplification-refractory mutation system (Tetra ARMS) PCR. Statistical data was analyzed by using SPSS software (version 22.0). Clinical characteristics of diabetic patients with and without foot ulcer were documented along with ethnically matched healthy controls. Results of genetic and statistical analysis showed that diabetic patients having TT genotype of SNP *rs1024611* exhibited significantly more prevalence of diabetic foot ulcer as compared to healthy controls. Results show that T allele of *rs1024611* is a risk allele which confers susceptibility to foot ulcer in Pakistani type 2 diabetes patients.

INTRODUCTION

Type 2 Diabetes (T2DM) is worldwide problem. In Asia, Africa and Europe the incidence is 5.5%, 7.2% and 5.1% respectively (Zhang *et al*.,2017), whereas in Pakistan, incidence is as high as about 11.77% (Ali *et al*., 2001), which may be due to custom of consanguineous marriages and environmental factors. There are many complications in Type 2 diabetes and foot ulcer is one of the serious and long term complication among them (Adeghate *et al*., 2006). Diabetic foot ulcer can cause damage to nerves, blood vessels, skin, and tendons and even bone (Su *et al*., 2018). Which may lead to insufficient blood circulation at wound site. In diabetic patients, neurotrophic ulcers are the most common lesions which may lead to amputation of the lower extremities in severe cases (Hokkam, 2009). Diabetic foot ulcer frequency differ from population to population varies from 13% to 3% in North America and Oceania, respectively (Boulton, 2000). Among Asian countries e.g., Pakistan has higher incidence of foot ulcer (15%) however, neuroischemic ulcer (59%) is most common than neuropathic (22.6%) and ischemic ulcer (18.3%) (Ashraf *et al*., 2011).

There are some genetic factors which may be responsible for development of T2DM neuropathy and foot ulcer. Genes like *MCP-1* related to inflamatory responses may be responsible for DFU development. *MCP-1* is monocyte chemoattractant protein which can activate macrophages lymphocytes and monocytes (Li, 2018). In hyperglycemic condition the production of *MCP-1* may increase in vascular endothelial cells. This abnormal condition may contribute toward the complication related to angiogenesis and vascular functions in diabetic patients (Jiang *et al*., 2016). In Chinese population enhanced production of *MCP-1* is reported among diabetic foot ulcer patients (Su *et al*., 2018; Dong *et al*., 2014). However,

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the effects of *MCP-1* rs1024611 polymorphism and its association with high level of *MCP-1* is rarely reported in diabetic patients.

Prevalence of type 2 diabetes with complication of foot ulcer is high in Pakistani population (Miyan *et al*., 2017; Kaimkhani *et al*., 2018). However, the genetics of the diabetic foot ulcer has not been explored yet. The purpose of present study was to investigate the effect of *MCP-1* rs1024611 polymorphisms on risk of diabetic foot ulcer in T2DM patients. This study may be helpful to access the risk of foot ulcer in diabetic patients and their targeted therapy.

MATERIALS AND METHODS

Enrollment of patients

This case control study was conducted from 2018 to 2019. Total 105 T2DM patients of DFU and 117 non- DFU were enrolled in this study. Hundred healthy individuals were randomly selected from same ethnic background, with same age and sex. All patients were enrolled according to standard inclusion and exclusion criteria of World Health Organization. Foot ulcer in T2DM patients was diagnosed by Wagner- Meggitt classification system (Cheng *et al*., 2010). All clinical features were recorded carefully e.g., fasting and random blood sugar level, urea and creatinine etc.

Genotyping

Five ml blood was collected from each participant and total genomic DNA was extracted (Grimberg *et al*., 1989). Primer sequences were design by Tetra ARMS primer design tool (http://primer1.soton.ac.uk/primer1.html).

DNA products were amplified in 10µl reaction, containing 1µl KCl buffer, 0.85µl $MgCl_2$,1µl dNTPs, 0.15µl Taq, 2.6 µl PCR water, 2 µl genomic DNA and 2 µl of two inner primers mixed with 0.4 µl of two outer primers. The thermal amplification program consisted of an initial denaturation at 95°C (2 min), followed by seven cycles at 95°C for1 min, 72°C for 1 min and 72°C for1 min, followed by thirty-three cycles at 95°C for 1 min, 65°C for 1 min and 72°C for 1 min and final extension at 22 °C for10 min. Products of PCR were analyzed in 2% gel electrophoresis.

The sequences of the Tetra ARMS PCR primers for MCP-1 gene rs1024611 SNP are as follows:

C allele ATCAGAAAGAAAGTCTTCTGGAAAGTTAC
T allele AGAGCAAGAAGTGGGAGGCAGACAGATA
Forward outer TATAGACATCCTGTGGCATGACCACTT
Forward reverse CTCCAAGCCAAATGCATTCTCTTCTAC

Statistical analysis

Statistical analyses were carried out using Minitab software version 18. Equality of proportion is tested using normal approximation. *P* value ≤0.05 was considered as statistically significant.

RESULTS

It was observed that diabetic patients with foot ulcer had age range of 36 to 75 years with average age of 51±0.99 years. Average age of male patients was 54±0.24 and female patients was 50±2.06, respectively. It is found that foot ulcer is common in age ranges between 45-60 years and most likely to develop in patients having family history of diabetes.

Table I. Clinical features of diabetic type II patients without and with complication of diabetic foot ulcer (DFU).

Parameters	Non-DFU N= 117	DFU N= 105	Control N= 100
BSR (mg/dl)	212± 2.97	254 ± 3.24	140±4.35
BSF (mg/dl)	228 ± 4.35	222 ± 2.23	95±2.25
Creatinine (mg/dl)	0.94 ± 0.03	1.73 ± 0.15	0.76± 1.25
Cholesterol (mg/dl)	169 ± 1.72	196 ± 5.0	165±1.22
Triglyceride (mg/dl)	145 ± 2.08	207 ± 11.6	140±1.35
LDL (mg/dl)	117 ± 1.21	135 ± 8.60	94±2.35
HDL (mg/dl)	37 ± 0.39	50 ± 2.46	60±3.24
Urea (mg/dl)	23 ± 0.64	35.9 ± 2.1	16±1.2
HbA1C (%)	7-9%	9-11%	3-4%
Systolic blood pressure	136 ± 0.93	138.3 ± 1.37	115±0.93
Diastolic blood pressure	92 ± 0.39	91.1 ± 0.68	75±0.97
BMI (Body mass index)	27 ± 0.43	29.7 ± 0.611	23±0.52

BSR, blood sugar random; BSF, blood sugar fasting; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, hemoglobin A1c

Clinical features are presented in Table I. It was observed that frequency of amputation of fore, mid, hind foot and complete foot in these patients was, 21%, 30%, 13%, 16% and 20%, respectively. Neurotropic foot ulcer was most common type than other types. Results of Tetra ARMS PCR showed that out of 117 of diabetic cases without foot ulcer, 35% patients carried TT, 21% CC and 42% carried CT allele. While, in foot ulcer patients (105) 47% cases showed TT, 26% CC and 29% carried CT alleles, while healthy controls showed TT genotypes in 19% individuals. Genotypic frequencies of different alleles in T2DM (DFU and non-DFU) and healthy controls as shown in Table II and showed that TT alleles

Table II. Distribution of allele frequencies of the SNPs *rs1024611* in diabetic patients with (DFU) and without foot ulcer (non-DFU) along with healthy controls (C).

Geno types	DFU n=105(%)	Non DFU n=117(%)	C n=100(%)	P-value CI (95%)		
				DFU/C	DFU/Non-DFU	Non-DFU/C
CC	09(8.6)	19 (16.2)	32 (32)	0.000* (-0.234; -0.145)	0.040* (-0.077;-0.005)	0.003* (-0.158;-0.063)
CT	47 (44.8)	51 (43.6)	45 (45)	0.486(-0.002;0.112)	0.430 (0.0117; -0.098)	0.417 (-0.014;0.097)
TT	49 (46.7)	47 (40.2)	23 (23)	0.000*(0.237;0.131)	0.164 (0.065;-0.044)	0.003* (0.172;0.070)
C	(0.30)	(0.38)	(0.45)	0.013*(-0.150;-0.039)	0.115(-0.080;0.030)	0.157 (-0.070;0.044)
T	(0.69)	(0.61)	(0.54)	0.014* (0.15;0.038)	0.117(0.080;-0.031)	0.158 (0.070;-0.045)

*Significant at 5% level of significance; CI= Confidence Interval for difference between proportions

are associated with DFU susceptibility and might be risk alleles of the disease. It can be observed statistically that proportion of patients with DFU and non-DFU having CC genotype of SNP rs1024611 are significantly less (p-value<0.05) as compared to healthy controls while TT alleles are significantly associated (p-value<0.05) with susceptibility of diabetic foot ulcer in Pakistani population as shown in Table II.

DISCUSSION

In this study association of *MCP-1* rs1024611 polymorphism with susceptibility of DFU in T2DM Pakistani patients was investigated. It was found that in Pakistani population male are more effected with DFU than female patients. Whereas in some population e.g. In Jordan, both sexes are equally affected with DFU (Jbour *et al*., 2003). Various factors may play role in development of foot lesions in diabetic patients. These factors include smoking, hormones, the prevalence and severity of vascular disease, and neuropathy etc. (Lim *et al*., 2017). In addition, it was found that females are less affected with sensory neuropathy and peripheral vascular diseases than males and same observation was evaluated in British population (Moxey *et al*., 2011). Which may be due to hormonal changes and other environmental factors which may involve different disease pathogenesis in both sexes (Yu *et al*., 2004).

Results of this study showed that higher HbA1c level was found among both groups while other clinical parameters e.g., creatinine, cholesterol, triglyceride, LDL, urea was significant in DFU patients. In Bengali and Chinese population, the level of creatinine, cholesterol, triglyceride, LDL, urea was also significantly higher in DFU patients than that of diabetic patients (Parial *et al*., 2013; Li, 2018). There are many age-related changes e.g., carbohydrate metabolism that interact with genetic background of the individuals which can be differ in different ethnicity.

This study showed the average value of BMI was 27±0.43 in diabetic patients without foot ulcer and 29±0.61 in DFU patients ($P < 0.05$). Therefore, there was significant difference between diabetic patients with and without foot ulcer in terms of BMI and risk for DFU. However, many studies reported that there is high risk of for foot ulceration in greater BMI (body mass index) of the diabetic patients (Khalifa, 2018). Which may be due to the effect of body weight on the planter pressure, although association is not consistent in some populations.

In Pakistani DFU population neurotrophic ulcer is more common than others types of ulcer as previously described in Chinese population and in Saudi population (Su *et al*., 2018; Obied *et al*., 2019). This may be due to the fact that patients with diabetes and severe peripheral arterial disease have increased susceptibility to sudden ischemia, which are resulting from arterial thrombosis that may increase the risk of ulcer and amputation. In developing countriesin DFU patients' frequency of amputation is 21% while in developed countries it may be low as 10-12% (Obied *et al*., 2019). This may be attributed to low hygienic and health status of developing verses developed countries. Most common reasons of higher amputation rate are poor debridement, severe infection, improper treatment, hyperglycaemia and unawareness of the patients (Li, 2018).

Some genetic factors play important role for developing DFU in T2DM patients. Monocyte chemo attractant protein -1 has a key role in developing susceptibility to DFU (Su *et al*., 2018). The *MCP-1* gene located at q12 position on chromosome 7 and SNPs *rs1024611* present in promoter region of *MCP-1* gene. *MCP-1* is monocyte chemo attractant protein which can activate macrophages lymphocytes and monocytes and slow the process of wound healing in diabetic patients (Li, 2018; Parial *et al*., 2013). It also regulates the inflammatory response and production of inflammatory cytokines. Results of this study showed that frequency of TT alleles (45%) is higher in Pakistani diabetic patients with DFU.

However, in Chinese population it was found that AG and GG genotype were more likely to develop DFU and GG is considered risky alleles (Su *et al*., 2018; Li, 2018). While, in Iraqi population, frequency of AG and GG genotype is also high in DFU patients (Obied *et al*., 2019). Results of different genetic associations of alleles with disease in different populations show the genetic heterogeneity of this disorder.

Increased level of *MCP-1* was observed in diabetic patients which is similar to the previously reported studies. Present study explores that T allele of *rs1024611* SNP is associated with enhanced *MCP-1* expression as reported in previous study. It may be due to allelic variants which might the function of gene, which may lead to abnormal immune response of the disease.

It is suggested that diabetic patients carrying TT alleles of SNP *rs1024611* of *MCP-1* gene were more likely to develop diabetic foot ulcer. This SNP might play important role in the development of DFUs in diabetic patients via regulate the process of gene transformation.

CONCLUSION

It is suggested that diabetic patients carrying TT alleles of SNP *rs1024611* of *MCP-1* gene were more likely to develop diabetic foot ulcer. This SNP might play important role in the development of DFUs in diabetic patients via regulate the process of gene transformation.

DECLARATIONS

Ethical statement

This study was approved by ethics committee of the Department of Biotechnology Lahore College for Women University, Lahore, Pakistan. Written informed consent was obtained by each participant. All the procedure were in accordance with Declaration of Helsinki.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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