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Full Length Article

Advanced Glycation End-products and Foot Ulceration in Type 2 Diabetic Patients: A Case Control Study

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ABSTRACT

A case-control study was conducted to determine association between foot ulcerations and advanced glycation endproducts (AGEs) among 1782 diabetic patients (type 1=124; type 2=1658) in Faisalabad, Pakistan. Active foot ulcers were identified in 3.75% of the patients. Sixty-seven patients with foot ulcers (49 males, 18 females) and 1715 patients without foot ulcers (854 males, 861 females) were evaluated for various clinical factors probably associated with foot ulcers. Diabetic patients with foot ulcers had longer duration of diabetes, poor glycaemic control and elevated plasma AGEs than patients without foot ulcers. There were no differences in renal, visual impairment, peripheral neuropathy and peripheral vascular disease between both groups. Patients with foot ulcers had plantar calluses, hallux valgus deformity and discolored nails. Multivariate analysis indicated that major independent risk factors of developing foot ulcers were duration of diabetes, peripheral neuropathy and glycaemic control but not AGEs levels. Despite its limitations, this study showed that diabetic foot ulcer is a multi-organ complication and emphasizes need for lifelong surveillance of the diabetic foot.

Key Words: Prevalence; Foot ulcers; Diabetes mellitus; Risk factors; Faisalabad

INTRODUCTION

Diabetes mellitus is one of the most common endocrine disorders affecting millions of individuals. The global prevalence of diabetes was estimated to be 2.8% in 2000 and is projected to be 4.4% in 2030 (Wild *et al*., 2004). Common diabetic foot complications include neuropathy, infections, vascular disease and ulcerations. Diabetic foot ulcers (DFU), prevalent in about 15% of the people with diabetes are precursors to lower extremity amputations (Dalla Paola & Faglia, 2006). As the rates of recurrence of foot ulcers are accelerating, being greater than 50% after 3 years, diabetic foot diseases are emerging as a major financial penalty for the people with diabetes (Boulton *et al*., 2005). The risk for ulceration and amputation is much higher in diabetics compare to the non-diabetic population. The lifetime risk of a diabetic individual developing an ulcer is 15-40% and every 30s a lower limb is lost due to diabetic complications somewhere in the world. The life-threatening and prolonged disabling consequences of foot ulcers contribute to social, psychological and financial consequences (Rathur & Boulton, 2007). However despite its dimensions, this growing burden remains frustratingly unrecognized in Pakistan. Management of foot complications is one of the core items in the amalgamation of economic and health resources addressed to the diabetic population (Dalla Paola & Faglia, 2006).

Pakistan is a South-Asian country with 160 million population. A review of literature indicates a rising trend in the incidence of diabetes mellitus in recent decades in Pakistan. The WHO estimates that by 2025, Pakistan will account for more than five million new cases of diabetes (Basit & Williams, 2006). More than six million people in Pakistan are living with diabetes (Rathur & Boulton, 2007). Diabetic Association of Pakistan in a study involving 500 people with diabetes has documented 20-40% prevalence of retinopathy, nephropathy and neuropathy (Shera *et al*., 2004). Ongoing rise in diabetes and related complications will promote pivotal events of diabetic foot ulcerations, but very limited information is available on diabetic foot amputations in Pakistan. Studies on DFU in Pakistan are very few and restricted. Some researchers reported only 1-2% incidence (Hashim *et al*., 1999; Rooh-ul-Muqim *et al*., 2003). Others reported 42% neuropathic and 58% neuro-ischaemic ulcers (Ali *et al*., 2001). Main risk factors for diabetic foot problems in Pakistan were lack of awareness, poor glycaemic control and duration of diabetes (Hashim *et al*., 1999; Ali *et al*., 2001).

Pakistan is least prepared to cope with this pandemic. Lack of resources and basic infrastructure are preventing access to screening, early diagnosis, treatment and palliative care. Diabetes and its complications arise from elevated plasma protein glycation levels and are associated with various viral infections (Riaz ul Hassnain *et al*., 2007; Saeed & Sheikh, 2008).

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Treatment imposes economical burden (Ali *et al*., 2008) and the documented incidence is quite ambiguous. A comprehensive understanding of risk factors for DFU would help clinicians to categorize patients by their risk status and schedule interventions accordingly. Faisalabad is the third largest city of Pakistan with an estimated 4 million residents. Objective of this study was to assess association between AGEs and development of DFU to support the adoption of effective measures for the surveillance, prevention and control of diabetes and its complications.

MATERIALS AND METHODS

In this case-control study we reviewed medical records of randomly recruited 1782 diabetic patients attending Out-Patient-Departments (OPDs) of District Headquarter Hospital and Allied Hospital, Faisalabad, Pakistan during June 2006 and July 2008. Before enrollment, ethical approval from institutional Advanced Studies and Research Board was secured.

The diagnosis of diabetes was based on WHO criteria (fasting plasma glucose≥7.0 mmol/L [126mg/dL] or post-prandial plasma glucose≥11.1mmol/L [200mg/dL]). The quintessential assessment of every patient was a comprehensive history, physical examination and the use of laboratory protocols to arrive at a differential or specific diagnosis. Following informed consent, case participants were given a physical examination and interviewed for exposure variables. Demographic data included age, sex, type of diabetes, duration of diabetes and type of treatment taken by patients (diet, oral hypoglycemic agents, insulin & combined). The presence of renal diabetic complication was assessed using following standards: no albuminuria (<20 µg/min.) vs. microalbuminuria (20-200 µg/min) (Savage *et al*., 1996). An ophthalmologist using fundoscopy through dilated pupils performed eye examination. Diabetic retinopathy was established as background vs. proliferative (Klein, 1988).

Dermatological, vascular, neurological and musculoskeletal systems were physically examined by two certified physicians. The dermatological examination included visual inspection of skin on legs and feet (heel, between toes & toenails), mainly the dorsal, plantar, medial, lateral and posterior surfaces to evaluate cutaneous diseases more commonly related to diabetes (Perez & Kohn, 1997). Skin on the feet was compared to the skin on the arms for changes in color, pigmentation, texture and turgor. Active ulcer was defined as a full-thickness skin break at least to Wagner stage 1, occurring distal to the malleoli (Wagner, 1981). Lesions in which the epithelium appeared to be intact (infections with no entry portal, blister or unrelated skin disease) were also included. A vigilant history was obtained about past ulcerations or lower extremity amputations (LEA). In musculoskeletal inspection, diabetic patients with foot ulcerations were inquired about symptoms (numbness, burning sensation, crawling & tingling) and physically inspected for foot deformities (hammer/claw-toe, hallux valgus, flat foot), skin (dryness, heel crack, calluses, fissures) and nail (discolouration, ulcer, thickened, badly cut, ingrowing) condition.

Neurological inspection included both peripheral neuropathy (PN) and peripheral vascular disease (PVD). PN was described as nerve damage characterized by sensory loss, pain, muscle weakness and wasting in hands, legs or feet. Neuropathy was considered if there was persistent numbness, paresthesia, loss of a tunning fork tested sense of vibration, or failure to elicite knee and/or ankle jerk reinforcement (Claus *et al*., 1993). PVD is arteriosclerosis of the lower extremities, blood vessel disease relating to narrowing and hardening of the arteries that supply blood to legs and feet. Presence or absence of intermittent claudication was considered as diagnostic criterion (Rose & Blackburn, 1968). At the end of questioning, patients were informed about the impact of their perceptions on their health.

Plasma glucose (mmol L^{-1}), glycated hemoglobin A_1c (%) and plasma AGEs were measured by glucose oxidase method, colorimetric method (A_1c Kit Biosystem, Spain) and ELISA method, respectively. Results were expressed as mean±standard deviation (SD) or number (n) or percent (%) as appropriate. In preliminary analysis, continuous variables were divided into normal and abnormal groups. Student's t-tests, chi-square and Mann-Whitney tests were performed to compare continuous, categorical and ordinal number data for diabetic patients with and without foot ulcerations. The independent risk factors associated with the development of DFU were identified by multiple analysis of variance. The 95% confidence interval (CI) was calculated when required and statistical significance was defined as a P value<0.05. Statistical data analysis was performed using SPSS for Windows (version 14.0).

RESULTS

Study comprised of 1782 people with diabetes (type 1=124, type 2=1658) divided into two groups namely; diabetic foot ulcer group (DFUG) and diabetic group without foot ulcer (DG). The overall prevalence of active foot ulceration identified at screening was 3.75% (67 of 1782). The median duration of these ulcers was 6 to 18 weeks and Wagner grade classification was as follows: Grade 0 (skin intact) =1%, grade 1 (skin & subcutaneous tissue only) =17%, grade 2 (muscle, tendon or joint capsule) =57%, grade 3 (periosteum & bone) =25%. The prevalence of past ulceration was 0.44% (8 of 1782). Therefore, overall foot ulcer history prevalence was 4.19 (75 of 1782). Past history of lower extremity amputation (LEA) was 0.22% (4 of 1782).

Clinical characteristics for the participants are presented in Tables I and II. Nearly all of the patients had type 2 diabetes (DFUG 94%, DG 93%) and those with DFU were mostly males (73%). Majority of the patients in both the groups were married (DFUG 97%, DG 99%), urban

dwellers (DFUG 61%, DG 81%) and engaged in manual activities (DFUG 65%, DG 63%). Within both groups, low and middle class communities predominated with relatively less literacy rate. The differences in age, plasma glucose, plasma AGEs between DFUG and DG were statistically insignificant.

The mean duration of diabetes was notably higher ($P < 0.001$) in DFUG compared to DG. DFUG patients also demonstrated considerably higher ($P < 0.005$) HbA_1c levels as compared to DG, suggestive of relatively poor glycaemic control. The diabetic patients using diet only for blood glucose control was three times higher in DFUG than DG (18% vs. 6%). Oral hypoglycemic agents (OHA) were more commonly used by DG as compared to DFUG (47% vs. 15%). Contrary to that, DFUG participants were frequent insulin users than that of DG (48% vs. 25%). The prevalence of PVD (6% vs. 6.35%), visual [Background retinopathy 1.5% vs. 0.93%; proliferative retinopathy: 2.98% vs. 3.0%] and renal impairments (4.48% vs. 4.08%) were not statistically different ($P > 0.05$) between DFUG and DG, respectively. Peripheral neuropathy detected in 10.5% of DFUG subjects compared to 0.23% DG had noteworthy association ($P < 0.0001$) with the development of foot ulcers. Three common skin disorders often seen in diabetic patients are diabetic dermopathy, necrobiosis lipoidica diabeticorum and bullous diabeticorum.

Reddish-brown papules of diabetic dermopathy were asymptomatic in 2.62% DG patients and 2.98% DFUG patients. Hyperpigmented necrobiosis lipoidica diabeticorum plaques on the shins were prevalent in 1.5% and 1.74% DFUG and DG patients, respectively. Bullous diabeticorum had fluid-filled bullae presentation in 1.5% diabetics with foot ulcers compared to 1.28% diabetics without foot ulcerations. DFUG subjects were examined for foot, skin and nail alterations, which are often unrecognized and frequently caused by inadequate footwear (Table III). Regarding the foot defects, the prevalence of hallux valgus was 11%, whereas callus was identified in 33% foot ulcer patient. Majority of the DFUG participants complained about numbness (73%) and burning sensations (48%).

On multivariate analysis, we noted numerous significant associations. Longer duration of diabetes ($P < 0.001$), inadequate diabetes management ($P < 0.005$) and peripheral neuropathy ($P < 0.0001$) and not AGEs (P 0.458) increases the propensity of people with diabetes to develop DFU. Many associations were conspicuous in their absence. Peripheral vascular disease, nephropathy, retinopathy and advanced glacation endproducts were insignificantly related to development of foot ulcers.

Table I: Clinical characteristics of diabetic patients with and without foot ulcers

Clinical characteristics	With foot ulcers DFUG	Without foot ulcers DG	*P-value
Patients (n)	67	1715	
Sex (male/female)	49/18	854/861	
Age at time of enrollment (years)	55.7 ± 8.2	54.2 ± 7.6	0.433
Types of Diabetes (1/2)	4/63	120/1595	
Duration of diabetes (years)	13.6 ± 7.3	9.4 ± 4.2	0.001
Plasma glucose (mmol l^{-1})	15.5 ± 6.9	15.7 ± 8.8	0.871
HbA_1c (%)	12.13± 2.11	10.54 ± 1.32	0.005
AGEs (unit/ml)	9.34	8.77	0.458

Data are expressed as n, mean ± SD, or %± SD
*P- values obtained by Students t- test or chi-square test

Table II: Prevalence of diabetic complications in diabetic patients with and without foot ulcers

Disease	With foot ulcers DFUG cases (n)	Without foot ulcers DG cases (n)	*P-value
Microalbuminuria	3	70	0.45
Peripheral Neuropathy (PN)	7	4	0.0001
Peripheral Vascular Disease (PVD)	4	109	0.89
Diabetic Retinopathy			
Background	1	16	0.95
Proliferative	2	52	0.47
History of ulceration	5	3	
History of lower extremity amputation	3	1	0.99
Cutaneouse disease	2	45	0.98
Diabetic dermopathy	1	30	0.88
Necrobiosis lipoidica diabeticorum	1	22	0.99
Bullous diabeticorum			

Data are expressed as n (number)
*P value obtained by Mann-Whitney test

Table III: Clinical features of diabetic foot ulcers in DFUG patients

Symptoms	n
Numbness	49
Burning sensation	32
Crawling	19
Tingling	7
Skin	
Dryness	34
Heel crack	56
Calluses	22
Fissures	14
Foot deformity	
Hammer	1
Claw-toe	5
Hallux valgus	7
Flat foot	2
Nail	
Discolouration	11
Ulcer	3
Thickened	2
Badly cut	2
Ingrowing	1

Data are expressed as n (number)

DISCUSSION

Frequency of DFU in our study was similar to the findings of Alavi *et al*. (2009) but opposite to other studies (Hashim *et al*., 1999; Basit *et al*., 2004; Agbor Ndip *et al*., 2006). Generally people avoid physicians and consult local hakeems (homeophathic doctors) for cure. Medical advice is requested only when pain is intolerable. So the variation in percent prevalence of DFU may be attributed to different setting, social beliefs and lack of awareness about

complications. Most of the people in Pakistan walk bare feet or use inexpensive shoes that are uncomfortable or ill fitted (Babar & Hatcher, 2005a). Ethnicity might have an effect on amputation. Asian diabetic are less exposed to foot ulcers than Caucasians (Chaturvedi *et al*., 2001). Although almost equal proportion of males (50.67%) and females (49.32%) prevailed among 1782 participants, majority of DFUG patients were males (73% vs. 27%). Different studies have shown that DFU is more common in males than females (Basit *et al*., 2004). This can be justified by the fact that women in Pakistan are often affianced in domestic activities, while men are engaged in occupational activities. This study confirmed such preponderance of men.

Type 2 diabetes mellitus is common among 90% people with diabetes (33)**.** 94% DFUG patients in our study had type 2 diabetes (El-Nahas *et al*., 2008). A much higher prevalence of type 2 diabetes (99%) in foot ulcer patients was found previously (Ali *et al*., 2001). Difference in mean age of DFUG and DG patients at the time of enrollment was insignificant. Mean duration of diabetes since onset was noticeably different between DFUG and DG. Data analysis indicated that duration of diabetes was associated with the DFU formation. Thus this observation was in conformity with the national and international reports (Hashim *et al*., 1999; Basit *et al*., 2004; Al-Mahroos & Al-Roomi, 2007). Several lines of evidence indicated that elevated blood sugar impairs wound healing, by affecting white blood cell function and neuropathy or vice versa (Bagdade *et al*., 1978; The Diabetes Control and Complications Trial Research Group, 1993).

Various trials mentioned a decline in macrovascular complications in diabetic patients who were recipients of intensive therapy. Nonetheless such outcomes of improved diabetes control (The Diabetes Control & Complications Trial Research Group, 1993) are statistically trivial findings that warrant additional research. Another factor with DFU formation, noted by this study was elevated HbA_1c levels in DFUG than that of DG. Poor glycaemic control is a well-known (Hashim *et al*., 1999; Basit *et al*., 2004; Al-Mahroos & Al-Roomi, 2007) dynamic aspect that compounds LEA in diabetes.

Unexpectedly, conventional risk factors such as peripheral vascular disease, retinopathy, nephropathy and AGEs (McNeely *et al*., 1995), were not associated with foot ulceration in multivariate analysis. This variability may be due to variations in the study designs established cultural belief in consanguinity in the population, over and above a genetic predisposition may have influenced contributions of these factors. Racial disparity in amputation incidence is well recognized (Chaturvedi *et al*., 2001). Neuropathy was lower in our study than in other reports, which indicated up to 23.7% prevalence (Agbor Ndip *et al*., 2006) but in accordance with another research (Bagdade *et al*., 1978). This could be due to late screening after the disease is apparently manifested and lack of proper assessment of neuropathy. Monofilament insensitivity, vibratory perception threshold testing, neuropathy disability score have shown to be effective tools but such assessment amenities are rarely available at public sector health care facility in Pakistan, which could have influenced the results. As expected, the presence of peripheral sensory neuropathy was a strong risk factor for foot ulceration in this study. It is irrefutable that the presence of peripheral neuropathy leads to the formation of foot wounds, as diabetic patients lose protective sensation. The results are consistent with studies conducted worldwide (McNeely *et al*., 1995; Hedetoft *et al*., 2009) that have shown neuropathy to be a pivotal risk factor for both amputation and ulceration in persons with diabetes mellitus. It is argued that neurological evaluation must be initial criteria to screen diabetic patients.

The factors determining the health behaviors in Pakistan may be seen in various contexts; physical, socio-economic, cultural and political (Babar & Hatcher, 2005a). Higher percentages of DFUG patients neither cared for their feet nor used proper footwear. Cultural beliefs and practices lead to self-care or home-remedies and consultation with traditional healers. Traditional or spiritual healers, clergymen, hakeems, homeopaths, or even quacks are often consulted for alternative therapies. The rational to attain traditional medicine is proximity, affordable fee, availability family pressure and strong community opinion (Babar & Hatcher, 2005b). Long-held misunderstandings continue to contribute to the national neglect of this pandemic. Community based activities can preserve and share valuable traditional knowledge.

Limitations of our study include dearth of common assays like BMI, blood pressure, blood urea, creatinine, total cholesterol, triglycerides, ankle brachial index, peak plantar pressure, vibration perception threshold. Hospitals are ill prepared to address these requisites and to cop with country's growing diabetes burden. It is argued that research is essential to underpin more informed policymaking in complex medically pluralistic countries. We should have analyzed correlation between treatment type and DFU formation.

Government with help from the international community must establish effective and affordable behavioral health endorsement diabetes control programmes by integrating clinical and public health systems if they are to fight the onslaught of diabetes mellitus.

In conclusion, results suggest that longer duration of diabetes, neuropathy and poor glycaemic control are more significantly associated with the presence of foot ulceration.

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