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Characteristics of bacterial infection in patients with diabetic foot and its relationship with degree of vascular lesions in lower extremities.

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Abstract

Objective: To analyse the infection characteristics of pathogenic bacteria in diabetic foot infection and its relationship with the degree of vascular lesions in lower extremities.

Methods: 103 cases of patients with diabetic foot complicated with infection admitted to our department from 2009 to 2014 were collected. During hospitalization, all patients underwent double lower limbs CTA or lower extremity angiography examination, and were divided into 4 groups according to the classification (A~D) of Trans-Atlantic Inter-Society Consensus (TASC). χ^2 test was adopted to analyse whether there was a difference in pathogenic characteristics and drug resistance between different TASC groups. Single factor variance analysis was used to clear and definite whether there were differences between different TASC groups.

Results: There were differences in the comparison of age, duration of diabetes, ABI, TBI, percentage of neutrophils between groups, which were statistical significance (t=14.36, P=0.000; Z=30.88, P=0.001; χ^2=6.76, P=0.000; χ^2=11.56, P=0.006; t=56.16, P=0.039). Among the comparisons of TASC groups, with increased degree of lower extremity vascular lesions, proportion of mixed infection was gradually increased, and there were significant differences in the incidence of mixed infection (P=0.004), besides, among patients with grade D, mixed infection cases accounted for 66.7%.

Conclusion: Mixed infection is significantly related to degree of lower extremity vascular lesions in diabetic foot, and with increased degree of lower extremity vascular lesions, proportion of mixed infection is gradually increased.

Keywords: Diabetes foot, Bacteria, Infection, TASC classification, Lower extremity vascular lesions.

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Introduction

Diabetic foot ulcer (DFU) is currently one of the main reasons for the hospitalization treatment of diabetes patients [1]. The occurrence of diabetic foot is closely related to nerve, vascular diseases and infection, which can lead to changes of the biomechanical properties in pelma of patients [2], symptoms of which include intermittent claudication, ischemic rest pain, ulcers and gangrene. Foot wounds are an increasingly common problem in people with diabetes and now constitute the most frequent diabetes-related cause of hospitalization [3]. People with diabetes have about a 25% chance of developing a foot ulcer in their lifetime, about half of which are clinically infected at presentation [4]. There are many factors for poor prognosis of diabetic foot, such as the elderly, male, heart failure, end-stage renal disease, larger area of foot ulcer, peripheral neuropathy, peripheral vascular diseases, infection and so on [5]. Among them, the lower extremity vascular disease and infection are the most important factors affecting the diabetes foot. In diabetic foot ulcer, infection is an important factor which influences prognosis, and even one of the immediate causes leading to amputation and death [6]. But the relationship between infection type of foot ulcer as well as pathogen and vascular lesions is still not clear. Therefore, we aimed to investigate the pathogen infection characteristics in foot infection patients and its relationship with the degree of lower extremity vascular lesions and provide guidance for the healing of diabetic foot ulcers among patients with vascular lesions in diabetic foot infection confirmed by angiography.

Objects and Methods

Research objects

103 cases of patients clearly diagnosed with diabetic foot infection and positive secretion culture and hospitalized for treatment in department of hypertension and endocrinology in Daping Hospital of Third Military Medical University from March 2009 to March 2014, of which there were 69 males and 34 females with age of (67.56 ± 11.31 y old), 8.0 (3.0, 15.0 y) history of diabetes, and 2.0 (0.55, 10.20 months') history of

diabetic foot. Diabetic foot patients with no infection were excluded.

Methods

Clinical data collection: The selected patients received medical history inquiries, physical examination, completion of admission assessment records, comprehensive evaluation and preliminary diagnosis and treatment program conducted by medical staff in the center during the period of hospitalization. Cross-sectional study was applied in this study. During hospitalization, all patients underwent double lower limbs CTA or lower extremity angiography examination, and were divided into 4 groups according to the classification (A~D) of Trans-Atlantic Inter-Society Consensus (TASC) [7].

Laboratory index: In the early treatment in the center, all patients received the monitoring of blood pressure, blood routine, liver and kidney function, blood lipids (total cholesterol, glycerin trilaurate, high density lipoprotein cholesterol, low density lipoprotein cholesterol), urine routine, 24 h urine, glycosylated hemoglobin (HbA1c), foot ulcer secretion culture and drug sensitive test and so on. Among them, foot ulcer secretion collection: after wound cleaning and debridement for the first time admitted to hospital, tissues with deep infection were excised with sterile instruments, placed in a sterilization capped container and brought to inspection in ^{1}H. Pathogenic identification: pathogenic culture was operated in strict accordance with the explanation of 'National clinical laboratory operation rules, and qualified specimens were directly inoculated on the blood agar plate by streak separation method. After incubation at 37°C for 24~48 h, bacterial colonies were identified for mycobacterium by micro biochemical dilution method. Drug sensitivity reports were performed in strict accordance with the latest standards recommended by the 'American clinical laboratory standardization committee' (NCCLS). The susceptibility analysis was identified by the bacterial lab and analysed as sensitive, intermediate, and resistant.

Severity classification of diabetic foot ulcer According to lower extremity artery angiography examination, lower extremity vascular CTA examination (equipment in CTA center was 'PHILPS BrillianceiCT256 layer') and so on, stenosis degree and lesions length of lower extremity vascular were cleared and defined to determine the classification. The classification standards of Trans-Atlantic Inter-Society Consensus (TASC) standard were adopted [8], which divided the classification into 4 types of A-D, and patients were divided into 4 groups according to the above.

Classification of infection degree Low-grade infection: Fester, redness and swollen, pain, hyperaesthesia, increased skin temperature, 2 or more than 2 tubercles, or the diameter of ethmyphitis, redness and swollen of ulcer margins<2 cm, while infection is limited to the skin and superficial subcutaneous tissue with no other local or systemic complications; moderate infection: general condition of patients includes stable glucose metabolism with 1 or more following symptoms or signs of infection: cellulitis whose diameter is >2 cm, lymphangitis, spread of sub fascial infection, abscess, gangrene of deep tissues (such as muscle, tendons, bones, joints, etc.) appearing; severe infection: a systemic infection symptoms or metabolism function disorders, such as fever, chills, confusion, etc. [9].

Statistical methods

SPSS19.0 statistical software was used. Measurement data were expressed by $\bar{c} \pm s$ or interval of Median and Quartile (MQ), single factor Analysis of Variance (ANOVA) or non-parametric test was adopted for comparisons of multi groups, and rate was expressed by count data, χ^2 test (Chi-square test) was used for comparisons of rate in multi groups.

Ethical consideration

The study was carried out in compliance with the Declaration of Helsinki of the World Medical Association, and according to a protocol approved by the Ethical Committee of Daping Hospital, Third Military Medical University, the approval number is 2009004. The objectives of the study were explained to the study participants and verbal consent was obtained before interviewing each participant.

Results

The influence of different factors on lower extremity vascular lesions of diabetic foot patients complicated with infection

According to the TASC classification, patients were divided into 4 groups, which were respectively 23 cases of grade A, 16 cases of grade B, 38 cases of C grade, and 26 cases of D grade (Table 1). There were differences in the comparison of age, duration of diabetes, ABI, TBI, percentage of neutrophils between groups, which were statistical significance (t=14.36, P=0.000; Z=30.88, P=0.001; χ^2=6.76, P=0.000; χ^2=11.56, P=0.006; t=56.16, P=0.039).

Infection characteristics of pathogenic bacteria

Based on the infection degree for classification, in diabetic foot patients with infection collected by this center, there were 38 cases of low-grade infection (36.9%), 46 cases of moderate infection (44.7%), and 19 cases of severe infection (18.4%). In low-grade infection, there were 21 cases of classes C and D patients (55.2%); in moderate infection, there were 30 cases of classes C and D patients (65.2%); in severe infection, there were 13 cases of classes C and D patients (68.4%). With the aggravated degree of vascular lesions, the ratio of above moderate infection was increasing, but there were no significant difference between groups. According to the drug sensitivity test, the results indicated the presence of antibiotic resistance accounted for 61.1% in total; there were 21 cases indicated with poor prognosis by double examination of blood routine before discharge after the infection was not controlled during hospitalization.

Classification according to pathogenic types

Single infection included gram-positive bacterium infection accounting for 29.6%, gram-negative bacterium infection accounting for 27.1%, fungal infection accounting for 4.8%, mixed infection accounting for 38.5%. 139 strains of pathogen were cultured, including 24 strains of *Staphylococcus aureus* accounted for 17.3%, 17 strains of *Pseudomonas aeruginosa* accounting for 12.2%; 10 strains of *Enterococcus faecalis* accounting for 7.2%, respectively covering the top 3. In addition, other *Staphylococcus, Streptococcus, Klebsiella pneumoniae* and *Escherichia coli* were also common. Multi-sample χ^2 test analysis showed that among the comparisons of TASC groups, there were differences in the ratio of mixed infection in pathogenic type, which had statistical significance (P=0.004). With the aggravated degree of lower extremity vascular lesions, mixed infection proportion was gradually increased, and among patients with grade D, mixed infection accounted for 66.7%.

Table 1. *Basic clinical data of 103 patients.*

TASC classification	n	Male/female (case)	Age x̄ ± s, y old)	Duration of diabetes (MQ, y)	Duration of DFU (MQ, months)	Length of hospital stay (MQ, d)	Systolic pressure (x̄ ± s, mmHg)	Diastolic pressure (x̄ ± s, mmHg)
A	23	16/7	57.61 ± 12.06[b]	5.00 (2.00, 8.00)[b]	2.00 (0.23, 12.00)	12.0 (10.50, 17.50)	131.48 ± 21.49	73.13 ± 11.13
B	16	09/7	66.88 ± 10.33[b]	7.00 (1.00, 11.75)[b]	1.50 (0.40, 15.50)	13.50 (8.75, 23.25)	142.94 ± 25.26	75.31 ± 15.92
C	38	26/12	69.92 ± 9.50[b]	14.00 (6.25, 20.00)[b]	2.00 (0.50, 10.25)	12.00 (9.00, 15.00)	142.61 ± 21.35	77.68 ± 19.10
D	26	18/8	73.35 ± 7.83[b]	8.50 (3.25, 17.25)[b]	3.00 (0.85, 8.00)	13.00 (9.00, 23.00)	146.27 ± 19.37	76.15 ± 9.78
TASC classification	ABI (x̄ ± s)	TBI (x̄ ± s)	HbA1C	Hemoglobin (x̄ ± s, g/L)	Leucocyte count (MQ) ×109/L	Neutrophile granulocyte (x̄ ± s%)		
A	1.04 ± 0.20[b]	0.78 ± 0.11[b]	9.52 ± 2.53	124.52 ± 23.37	7.04 (5.80, 9.71)	69.41 ± 11.99[a]		
B	0.71 ± 0.8[b]	0.47 ± 0.21[b]	9.63 ± 1.68	119.44 ± 13.82	9.76 (6.36, 13.33)	77.99 ± 11.61[a]		
C	0.77 ± 0.30[b]	0.50 ± 0.32[b]	9.27 ± 2.11	116.42 ± 19.47	8.65 (6.94, 12.00)	74.34 ± 11.55[a]		
D	0.51 ± 0.38[b]	0.41 ± 0.34[b]	10.04 ± 2.76	115.98 ± 28.32	9.63 (7.66, 12.78)	77.70 ± 7.95[a]		
TASC classification	AST (x̄ ± s, U/L)	ALT (MQ, U/L)	Scr (MQ, μmol/L)	Total cholesterol (x̄ ± s, mmol/L)	Glycerin trilaurate (x̄ ± s, mmol/L)	LDL-CL (x̄ ± s, mmol/L)	HDL-CL (x̄ ± s, mmol/L)	
A	19.42 ± 8.88	17.00 (12.00, 24.70)	69.15 (61.75, 85.55)	4.30 ± 1.10	1.19 ± 0.46	2.50 ± 0.79	0.96 ± 0.31	
B	15.34 ± 4.83	13.30 (10.65, 20.43)	68.35 (61.15, 82.67)	4.42 ± 1.00	1.35 ± 0.83	2.58 ± 0.77	0.97 ± 0.33	
C	18.01 ± 7.79	14.50 (11.40, 19.40)	75.65 (64.95, 19.4)	4.37 ± 1.15	1.50 ± 1.12	2.83 ± 0.89	0.96 ± 0.27	
D	19.77 ± 11.06	16.45 (10.06, 21.65)	75.90 (64.15, 21.65)	4.10 ± 0.98	1.19 ± 0.46	2.37 ± 0.70	0.99 ± 0.31	

[a]P<0.05, [b]P<0.01, comparison among classifications

Discussion

The prevalence of diabetes in China has been increased year by year, and the compliance rate of treatment is low. The risk of chronic complications is high, of which diabetic foot covers all kinds of damage factors in diabetes, and due to the poor prognosis, the long-term quality of life in patients is seriously affected. In recent years, domestic and abroad research on related prognostic factors for the diabetic foot patients with infection has gradually increased. Some current research focuses on Wagner grading of diabetic foot and infection degree of foot ulcer as point of penetration, a number of demographic, clinical data, laboratory index information, etc. are selected; analysis of the above factors which affect the prognosis of diabetic foot infection are conducted [10-12]. In this paper, we use the TASC classification for analysis on the relationship between lower extremity vascular lesions and related clinical data, laboratory index, infection degree and pathogen of selected patients, and through the analysis on foot

ulcer secretion culture and results of drug sensitive test, infection characteristics of pathogen infection is confirmed, and its relationship with vascular lesions is analysed.

There are many factors that affect the prognosis of patients with diabetic foot, of which two of the most important ones are lower extremity vascular lesions and infection, and lower extremity vascular lesions in diabetes are characterized by lower knees vascular lesions. Diabetic foot ulcer is easily complicated with infection, the presence of infection will further aggravate the patient's disease condition, and two-way interaction will lead to deterioration during the late period of diabetic foot, amputation and even life-threatening. Domestic study [13] showed that the pathogens of diabetic foot patients with infection were mainly *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, which had high resistance rate to penicillin and cephalosporin. The foreign research [14] indicated that in foot infection patients, *Pseudomonas aeruginosa, Staphylococcus aureus, Colibacillus* were common, and the sensitivity to penicillin and cephalosporin showed a downward trend. This study found that the foot ulcer patients were mainly infected by *Staphylococcus aureus*, *Pseudomonas aeruginosa, Enterococcus faecalis* with more resistant strains of pathogenic bacteria, which was not consistent with the pathogenic bacteria reported in domestic and at abroad, suggesting that the infection of foot ulcer patients in this area was more serious, and had the characteristics of bacteriology in this region. Among the patients with diabetic foot infection included in this study, patients with a variety of antibiotic resistance accounted for 61.1% of the total, indicating that the proportion of drug resistance appearing in patients with foot infections was higher. In the period of hospitalization, after standardized treatment, there were 21 cases whose ulcer was not healed, and the clinical manifestation and the result of secretion culture still indicated that the infection had not been corrected (7 cases of non-drug resistance, 14 cases of drug resistance), suggesting that the prognosis of the patients with drug-resistant foot infection was poor. Further analysis showed that the infection was difficult to control, considering the ulcer site was often complicated with severe circulatory disorder caused by vascular lesion, which made the antibiotics difficult to reach the site of infection, leading to the blood concentration of antibiotics in the infection site decreased, and long-term use of antibiotic made incidence rate of resistant bacteria infection increase significantly. Foreign studies also showed that secondary infection of drug-resistant bacteria in diabetic foot was the main cause of final amputation[15,16]. This study also found that there was a significant difference in mixed infection among pathogenic types between the different groups of TASC. Among TASCA or class B patients with lower extremity vascular lesions, all types of bacterial infections were common; however, in TASC, classes C and D patients, the proportion of mixed infection increased gradually, and the proportion of mixed infection in D patients was up to 66.7%. With the increase of the severity degree of the vascular lesions, the proportion of mixed infection also increased, which suggested that the patients with high severity degree of lower vascular lesions often had multiple bacterial infections, leading to further deterioration of diabetic foot ulcer.

Diabetic foot ulcer complicated with infection not only affects the healing of ulcer surface, it also causes the inflammatory reaction which is a main factor affecting systemic body. The study found that in the comparison of each group by TASC classification, with the aggravation of tissue circulation ischemia degree, there were significant differences in neutrophil percentage, and the higher the classification of TASC, neutrophils participate in inflammation more significantly, suggesting that patients with serious lower extremity vascular lesions often tends to have serious infection. Neutrophils are the dominant player of inflammation and are involved in tissue damage, and they are at the forefront of resisting microbial pathogens (especially purulent bacteria) invading human tissues. When the infection of diabetic foot occurs, they are attracted to the site of inflammation by chemotactic substances. Because neutrophils contain a large number of lysosomal enzymes, it can decompose bacteria and tissue debris engulfed into the intracellular, and prevents the spread of pathogenic microorganisms in the body. The clinical manifestations of diabetic foot patients with infection are diverse, such as ethmyphitis, superficial infectious ulcer of skin (erysipelas), deep soft tissue infection (abscess, necrotizing fasciitis), osteomyelitis, gangrene, etc. [17]. High concentration of inflammatory factors is closely related to the difficult wound healing of foot ulcers [18]. Therefore, it is necessary to strengthen the anti-infective therapy and improve the revascularization in patients with foot infection, which may be an important means to improve the prognosis of diabetic foot patients.

The clinical significance of this study: mixed infection in diabetic foot has obvious correlation with the degree of lower extremity vascular lesions in diabetic foot, and with the aggravated degree of lower extremity vascular lesions, proportion of mixed infection gradually increases, thus improving vascular lesions are also part of procedures in infection control, especially the infection which is difficult to control may have lower extremity vascular lesions, which need strengthened screening and treatment. In addition, revascularization can effectively improve lower limb blood supply of patients with arterial occlusive disease, also helps to control infection, so as to slow down or prevent the further deterioration of diabetic foot ulcers [19].

Limitations of this study: this study is a retrospective analysis, which is failed to follow up long-term amputation and survival situation of patients, and the next step should be strengthening the follow-up observation of the above patients.

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