Original Article

Role of Polymorphonuclear Leukocyte in Diabetic Foot

1.Saba Fazle Rabbi 2.S.Mohsin Turab 3.Meh Jabeen 4. Shah Nawaz Jamali

1. Assoc. Prof. of Pathology 2. Assoc. Prof. of Pharmacology & Therapeutics 3. Assoc. Prof. of Physiology 4. Asstt. Prof. of Pharmacology & Therapeutics, Hamdard College of Medicine and Dentistry Hamdard University, Karachi.

ABSTRACT

Objective: To study the etiopathogenesis of foot injuries in patients of uncontrolled diabetes mellitus.

Study Design: Retrospective study of tissue samples received from diabetic patients clinically diagnosed as gangrene.

Place and Duration of Study: This study was conducted in the Department of Ophthalmology, JPMC, Karachi from July 2009 to June 2011.

Materials and Methods: 150 cases of uncontrolled diabetes mellitus with wounds of foot were included. 150 cases of known diabetics with peripheral neuropathy and history of loss of sensation were subjected to follow up of 2 years Follow up was done on the cases. As a first step blood sugar was brought under control and broad spectrum antibiotic was given. Wound debriment was done in all cases. The specimens were subjected to H/E and Gram's staining.

Result: In H/E, liquifactive necrosis, polymorphonuclear leukocyte, mononuclear cell infiltrate, few lymphocyte plasma cells & fibroblasts were seen. New blood capillaries were few or absent. Both gram positive and gram negative organisms were isolated. 79% were gram positive and 21% were gram negative.

Conclusion: Hyperglycemia causes relative anoxia in the micro environment of the tissue due to damage of peripheral neurons. Lack of adequate circulation leads to ischemia, which is super added by infection of the subcutaneous tissue. The resultant effect is liquifactive necrosis and complete lysis of tissue. Removal of such putrefied tissue is mandatory to stop further damage to the tissue.

Keywords: Diabetes mellitus, gangrene, staphylococcus aureus.

INTRODUCTION

Diabetes mellitus is a clinical syndrome characterized by hyperglycemia due to relative or absolute deficiency of insulin[1]. A complex called AGE is deposited systemically in different tissues. Uncontrolled hyperglycemia affects the peripheral nerve fibers which hampers the autonomous control of micro-capillaries in the peripheries. The mechanism of vasodilatation in warm environment and vasoconstriction in cold is due to the autonomic control of the peripheral capillaries. In diabetes the small capillaries remain in constant vasoconstriction and the microcirculation is reduced due to non-compliance of capillaries. [2]. The tips of distil phalanges are dependent on simple diffusion of nutrients, glucose and oxygen from viable and competent capillaries which is reduced and area becomes hypoxic. Due to lack of vasodilatation of venular end of the capillaries the transudate starts to accumulate in this area. The toxic waste matter like lactic acid, oxygen derived free radicals slowly starts to damage the tip of the fingers. Hence there is relative hypoxia from the beginning that finally results as ischemia [3]. Peripheral neuropathy also leads to the destruction of sensory neurons especially those of touch and pressure. This desensitization leads to minor foot injury which is missed at the initial phase and most of the time ignored by the patient. Minor cracks also appear in cold and dry climate which are the actual portal of entry for normal commensals.

The PML Polymorph nuclear leukocytes which are the first line of defense against normal commensals are sluggish and have a tendency to stick on the capillary wall. The chemotaxis is slow and they fail to reach site of injury and very few successfully phagocytose the bacteria. The most common bacteria found in diabetic foot are Staphylococcus aureus and Pseudomonas[4,5]. These bacteria thereby gain access to the subcutaneous tissue through the damaged skin. Here they multiply and produce enzymes/ toxins like coagulase/ leukocidin or have antigenic domain on their cell wall like Lipid A[6]. These microbiological chemicals entice polymorphonuclear leukocyte to the site of injury. The number of PML gradually starts to increase and a time comes when PML are the predominant cell observed at the site.[7] The MAO system of PML is designed to kill these bacteria but as their capability to phagocytose is greatly reduced in controlled diabetes the fail to destroy them. Absence of vasodilation ends in ineffective and trapped PML. Hence the inflammation becomes suppurative. This phenomenon is appreciated in histological sections of wound samples collected from diabetic foot [8]. The PML contain lysosomal enzymes like lipases, protinases DNAses which digest viable tissue. The type of necrosis seen in diabetic foot is therefore liquifactive instead of coagulative[9]. This type of necrosis provides platform for further bacterial

multiplication and they quickly replicate and later appear as necrotizing type[10]. The chemokines like VEGF and FGF are not secreted by the inflammatory cells and new capillaries are not formed which further aggravates the situation. Therefore until a lot of collagen, necrotic tissue and heavy bacterial growth and damaged capillaries are not removed, the routine medical management of hyperglycemia alone would be insufficient to treat such cases. It is therefore necessary to debride the wound. This decreases the bacterial load and removes favorable environment for their growth. The dead non-functional capillaries and permanently damaged tissue is removed which encourages nerve vasculiztion. The more this process is delayed the slower is the healing which later may result in amputation of a limb[11].

MATERIALS AND METHODS

Retrospective study carried out department of surgery, Jinnah Post Graduate Medical Centre, Karachi, consisting of 150 cases of known uncontrolled diabetics with diabetic foot injury over a period of 2 years. The contaminated wound samples were subjected to Haematoxyline and Eosine. All cases without foot lesions and all euglycemic patients were excluded from this study.

Table No.1: Percentage of Male and Female in Gram +ve & Gram -ve

Type of Bacteria	Male	Female	Total
Gram positive	81(54%)	38(26%)	119(79%)
Gram negative	20(13%)	11(7%)	31(21%)

Table No.2: Percentage of Male and Female in different microscopic features

Microscopic features	Male	Female	Total
Liquifactive necrosis	101	49	150 (100%)
PML	101	49	150 (100%)
Macrophages	17	03	20 (13%)
Fibroblast	16	06	22 (15%)
Lymphocytes	11	04	15 (10%)
Plasma cells	08	02	10 (7%)
New blood vessels	02	00	02 (1%)

RESULTS

Total number of patients enrolled was 150.Among them we isolated Gram positive cocci in 54% which were males and 26% females and Gram negative bacilli in 13% males and 7% females. It was noted that significant number of cases had Gram positive cocci infection that is 79% shown in table # 1.

With reference to table # 2 in all of 150 cases we observed infiltration with PML and liquifactive necrosis. There was presence of macrophages (13%), lymphocytes (10%), plasma cells (7%) and fibroblasts (15%). Only 1% of patient showed newly formed capillaries.

DISCUSSION

The incidence of gangrene is greater in patients of uncontrolled diabetics[12]. The factors responsible for its development are peripheral neuropathy and micro-vascular disease. There is loss of peripheral sensation and inflammatory process for minor daily injury[13]. This is further aggravated by secondary invasion of the site of injury by normal commensuals specially gram positive bacteria[14]. Complex of AGE is formed due to constant hyperglycemic condition. As there is no enzyme system to affectively neutralize AGE, it gets deposited within the microvasculature throughout the body[15]. The tip of the distil phalanges is dependent on its competent vasculature. Deposition of AGE reduces the flexibility of these vessels. Hence the environment becomes anoxic and favors ischemia. Venous return becomes sluggish and minor injury leads to cuts in the soft skin[16]. The normal commensuals get entry into the subcutaneous tissue and are not removed from the site quickly due to slow movement of PML[17]. These bacteria get time to multiply and secrete their enzymes and toxins which will fully recruit PML. In all of our 150 cases we observed heavy infiltration by PML. The stunted PML have lysosomal enzymes that degrade the normal tissue along with bactericidal activity. The tissues loose their shape and structure permanently and this type of necrosis is known as liquifactive necrosis[18,19]. We also observed that the type of necrosis evident was liquifactive necrosis in all of the cases. In diabetic foot there is predominance of acute inflammation however at places where there is chronic phase or little formation of healing tissue there is presence of other cells also, but their presence is numerically insignificant[20]. Lack of healing tissue suggests that until bacterial load and putrefied tissue is not removed the natural capability of the tissue to regain healing cannot be achieved. The most common microbes implicated in the pathogenesis of diabetic foot are the commensuals of the skin[21]. The gynogenic bacteria recruits PML and uses them to destroy the healthy tissue[22]. In our study the tissue section showed that the bacteria were surrounded by PML and Liquifactive necrosis in all 150 cases. Therefore it is mandatory to remove the putrefied tissue. Once wound debriment is done oxygen and nutrients can be restored by healthy capillaries and the bacterial load is reduced.

CONCLUSION

Since PML are the main defense against normal commensals, every effort must be made to diagnose such cases as early as possible so that the damaging effect of hyperglycemia on innate immunity is reduced and amputation of limb can be avoided. Timely

intervention and minor surgical debriment can improve the quality of life of such patients.

REFERENCES

1. Kahn R, Buse J, Ferrammini E. The metabolic syndrome: time factor for a critical appraisal. Diabetic care. 2005;18(9):2289-2304.
2. Vlassara H, Palace MR. Diabetes and advanced glycation end products. J Int Med 2002; 251(2): 87-101.
3. Gray M, Ratcliff C. Is hyperbaric oxygen therapy effective for the management of chronic wound? JWCN 2006;33(1):21-25.
4. Senneville E, Melliez H, Beltrand E. Culture of percutaneous bone biopsy specimen for diagnosis of diabetic foot osteomyelitis. Clin Infect Dis 2006; 42 (1):57-62.
5. Wieman TJ. Principal of management: the diabetic foot. Am J Surg 2005;190 (2):295-299.
6. Bansal E, Garg A, Bhatia S, et al. Spectrum of microbial flora in diabetic foot ulcers. Indian J Path & Microbiol 2008;51(2):204-208.
7. Ertugrul MB, Baktiroglu S, Salman S, et al. The Diagnosis of osteomyelitis of the foot in diabetics: microbiological examination is vs. magnetic resonance imaging and leucocyte scanning. Diabetes Medicine 2006;23(6):644-653.
8. Korzon A, Michael E. Role of microcirculation in diabetic foot ulceration. Int J Lower Extremity Wounds 2006;5(3):144-148.
9. Boyar P, Kremer G. Lysosomal membrane permeabilization in cell death. Oncogene 2008; 27: 6434-6451
10. Brem H, Sheehan P, Rosenberg H. Evidenced Based protocol for diabetic foot ulcers. Plastic and Reconstructive Surgery 2006;117(7S):193S-209S.
11. Cavangh PR, Lipsky BA, Bradbury AW, et al. Treatment of diabetic foot ulcers. The Lancet. 2005;366:1725-1735.
12. Lavery LA, Armstrong D, Robert P, et al. Risk factors for foot infection in individuals with diabetes. Diabetes Care 2006; 29(6):1288-1293.
13. Gerard S. Diabetic neuropathy- a review. Nature Clin Pr Neurology 2007; 3:331-340.
14. Goh SV, Cooper EM. The role of Advanced Glycosylation end product in progression of complication of diabetes. JCEM 2008;93 (4): 1143-1152.
15. Gupta A, Tripathi AK, Tripathi RL, et al. Advanced glycosylated end products mediated action of PML in diabetes Melliyus and associated oxidative stress. IJBB 2007;44 (5):373-378.
16. Fisher TK, Wolcott R, Wolk DM. Diabetic foot infections: A need for innovative assessments. Int J Lower extremity wounds 2010;9 (1):31-36
17. Slater RA, Lazarovitch I, Bolder Y. Swab culture accurately identify bacterial pathogens in diabetic foot wounds not involving bone. Diabetic Med 2004;21(7):705-709.
18. Armstrong DG, Benjamin A, Lipsky A. Advances in the treatment of diabetic foot infections. Diabetic Tech. & therapeutics 2004;6 (2):167-177.
19. Anderson CA, Roukis T. The diabetic foot. Surg Clin North America 2007;87(5);1149-1177.
20. Gadepalli R, Dhawan B, Sreenivao V, et al. A clinicopathological study of diabetic foot ulcers in an Indian tertiary care hospital. Diabetes Care 2006;29 (8):1727-1732.
21. Williams DT, Hilton JR, Harding KG. Diagnosing foot Infection in diabetes. Clin Infect Dis 2004; 39: 883-886.
22. Miyama S, Shirai A, Yamamoto S, et al. Risk factors major limb amputations in diabetic foot gangrene patient. Diab Res Cin Practice 2006;71 (3): 272-279.

Address for Corresponding Author:
Dr. Saba Fazle Rabbi,
Assoc. Prof. of Pathology,
Hamdard College of Medicine and Dentistry,
Hamdard University, Karachi.
Cell No. 0300-2219089