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ANIMAL MODELS FOR DIABETES GANGRENE: UNDERSTANDING THE ETIOLOGIES, PATHOGENESIS AND TREATMENTS

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ABSTRACT

Diabetes mellitus is one of the main problems that healthcare systems are now confronting. Diabetes patients may have a variety of issues, including diabetic foot ulcers. A common consequence of diabetes is diabetic foot ulcers. The complexity of diabetic mellitus has increased during the past several decades. According to study, diabetic foot ulcers are the most frequent reason for hospitalisation for diabetics. All forms of gangrene are brought on by a decrease in blood flow to a specific area. A few typical causes of gangrene include vascular problems, burns, Raynaud's syndrome, diabetes, injuries, immune system deficiencies, etc. The four elements of the diabetic gangrene pathogenesis are as follows Vascular, Neuropathy, Infection, and Delayed Wound Healing. The broad etiologies, pathophysiology, investigation, and treatment of diabetic gangrene are briefly reviewed in this paper. Additionally, we highlighted rodent models of diabetic gangrene like arachidonic acid-induced hind limb gangrene and reliable mouse model of hindlimb gangrene.

Keyword: Diabetes Mellitus, Diabetic foot ulcer, Animal Model

INTRODUCTION

One of the major issues facing healthcare systems today is diabetes mellitus (DM), a threat to public health that has grown significantly over the past two decades. Epidemiological studies show that there were roughly 30 million cases of diabetes mellitus in 1985, 177 million in 2000, 285 million in 2010, and it is predicted that there will be more than 360 million cases by 2030 if current trends hold **[1-2]**.

Multiple problems, including diabetic foot ulcers, can occur in DM patients (DFU). DFU is a frequent DM complication that has been rising over the past few decades. DFU is predicted to affect 15% of diabetes patients overall throughout the course of their lifetime. The prevalence of DFU ranges from 4% to 27%, notwithstanding the difficulty in obtaining precise statistics on this condition **[3-5]**.

Mobility may also start to be a difficulty at some point, which makes the situation worse. The primary consequences of limb amputation are closely connected. Amputation results in a lifelong impairment and the inability to carry out several daily tasks. In the absence of well-articulated solutions that are implemented at all levels, it makes sense to expect an increase in complications like DFU as the diabetes epidemic continues to expand. In order to guarantee that DFU is either avoided or recognized early enough, special attention to foot care should be a focus of patient education and management **[6]**.

Etiology:

A reduction in the blood supply to a particular location causes all types of gangrene to develop. Since the tissue is deprived of oxygen and nutrition, it dies.

Gangrene causes is vascular issues, cold, scalds, and severe burns, Raynaud's syndrome, diabetes, injury, dry gangrene, embolism, immune deficiency

Gangrene Risk factors include:

Smoking, Obesity, diabetes, high blood pressure, and other causes of vascular Disease, Excessive alcohol intake, which can lead to nerve damage, Impaired immune function, due for example, to HIV infection, Chemotherapy and radiation therapy Intravenous drug use Rarely, the anticoagulant medicine warfarin usage is associated with gangrene **[7]**.

Pathogenesis:

Neuropathy:

We still don't fully understand the precise process through which diabetes produces neuropathy. However, thanks to decades' worth of reliable data from clinical and animal investigations, key hints about how hyperglycemia results in neuropathy have been uncovered. The polyol pathway has been highlighted as a key contributor to diabetic neuropathy by reliable data. Aldose reductase converts excess glucose to sorbitol in this process, which is an alternative to the common glycolytic pathway, and sorbitol dehydrogenase then oxidizes the sorbitol to fructose.

In the context of hyperglycemia, this route substantially functions and has significant effects. These processes require both NAD and NAD as co-factors, and the competing use of NADPH compromises the cell's capacity to access reduced glutathione, a powerful antioxidant.

Reactive oxygen species cannot be sufficiently eliminated because glutathione levels are low. Thus, oxidative stress develops, impairing regular nerve function. Additionally, the buildup of metabolites causes a decrease in the production of the myoinositol needed for neural transmission in nerve cells **[8] (Figure 1)**.

Sign and Symptoms:

The major features of wet or dry gangrene are affected body part loses color, the region will initially seem discolored before drying up and becoming dark. In dry gangrene, the color will change from red to black, whereas in wet gangrene, the area will swell and have an unpleasant odor. Brownish pus that has a particularly unpleasant odor is produced by gas gangrene. Skin that appears shiny and sheds, with a distinct line separating the affected from the healthy skin. Pain is initially present, and then there is a lack of feeling and immobility. The area will feel chilly to the touch and the pulse in the arteries will stop.

Internal gangrene

Although significantly different, gangrene of the internal organs also involves tissue death. Internal gangrene could not show any outward symptoms, but septic shock and other problems could lead to the following: fever and chills, confusion, nausea, vomiting, and diarrhea, low blood pressure leading to light-headedness and fainting, shortness of breath and increased heart rate.

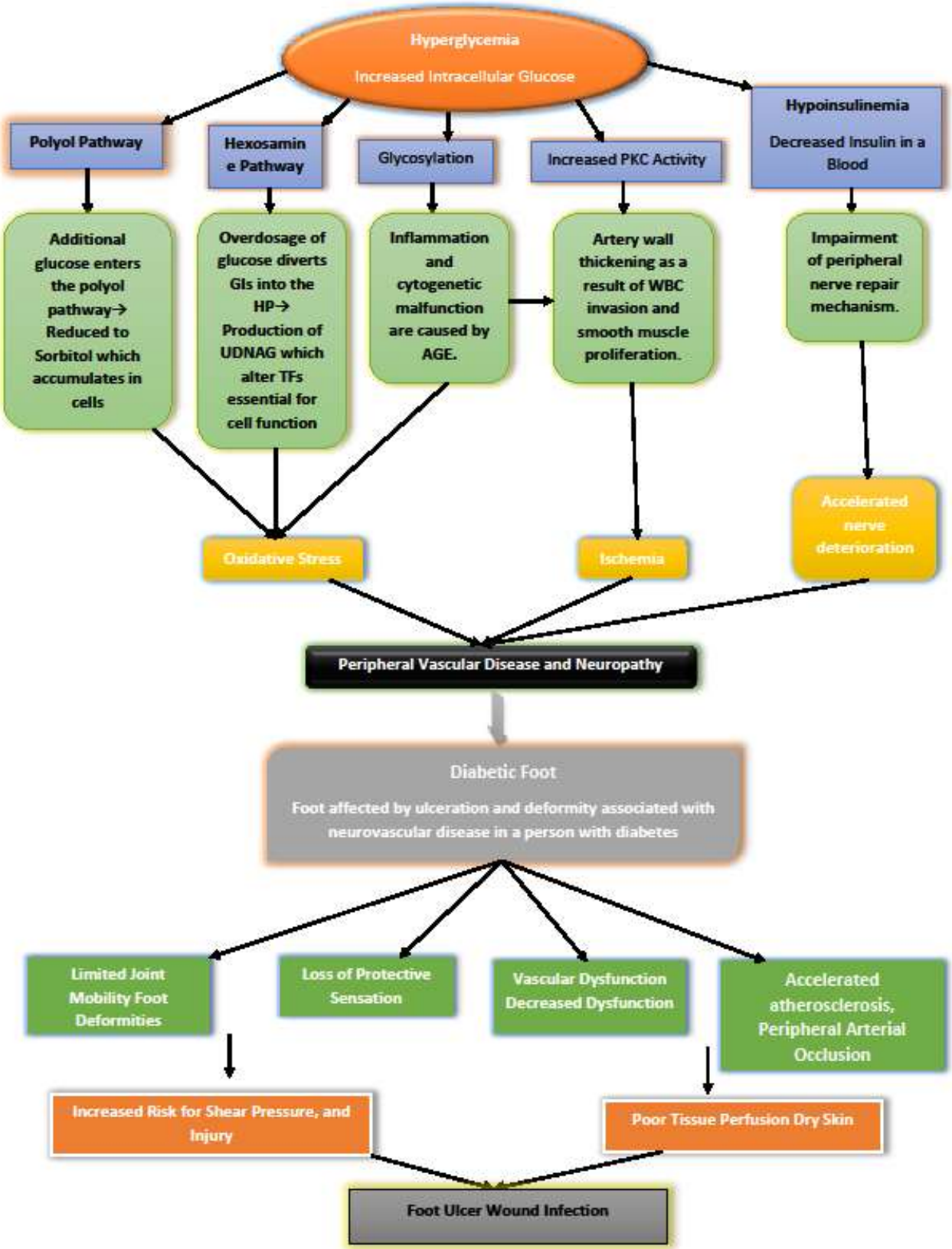

Figure 1: Pathophysiology of Diabetic Gangrene

Investigation:

The severity and complexity of the vascular disease will be determined by additional tests that are advised for ischemia gangrene, which will help focus treatment options. In a patient with tissue loss, non-invasive testing, such as an ankle brachial index (ABI), is crucial for the early detection of PAD; if it is less than 1.0, it is considered abnormal. When there is tissue loss, ankle pressure beyond 70 mmHg is regarded as abnormal, and ankle pressure between 40 and 60 mmHg is also consistent with critical ischemia **[9-10]**.

Additional imaging is done to pinpoint the lesion(s) if the ABI is consistent with vascular disease. Duplex ultrasonography, digital subtraction angiography, CT angiography, and MRA are all possibilities. Additional non-invasive testing options include ankle pressures, toe pressures, and transcutaneous oxygen pressures if the ABI is inconclusive, which is typical in diabetic individuals and those who are older since vascular compressibility may be impaired **[11]**.

Gram stain and wound cultures can assist identify the bacterial a etiology to help direct antibiotic therapy if gas or wet gangrene is suspected, but the diagnosis is normally made clinically. Due to the possibility of skin bacteria contamination, surface wound swabs are rarely effective. Instead, the sample should be collected through deep swabbing or aspiration of purulent discharge **[12]**.

Subcutaneous gas, which is invariably pathogenic and associated with gas gangrene and type I necrotizing skin infections, may also be visible on X-rays **[13-14]**.

Both MRI with aberrant signal intensity in the deep fascia and CT with contrast revealing a lack of fascial enhancement can aid in making the diagnosis. However, if there is clinical suspicion, surgical intervention should not be postponed. If the diagnosis is ambiguous, patients can be assessed using local exploration while under local anesthesia at the bedside. Necrotizing infections are characterized by the return of "dishwater" fluid and the ease with which fascial planes can be dissected **[15-16]**.

Treatment / Management:

The goal of treating ischemia gangrene is to increase blood flow in order to lessen pain during rest and heal ischemic wounds. It is doubtful that tissue can fully recover once ulcers have developed into dry gangrene, however tissue loss can be reduced with medicinal and surgical care. Antiplatelet therapy with aspirin or clopidogrel is one of the medical treatments for ischemic gangrene. Patients with diabetes should achieve adequate glucose control, ideally to a hemoglobin A1C less than 7 percent, and hyperlipidemia should be treated with a

statin as necessary. Quitting smoking is essential to lowering the risk of disease progression.

If there is severe necrosis of the weight-bearing section of the foot, refractory pain, sepsis/uncontrolled infection, paresis of the extremities, or a short life expectancy, primary amputation (amputation before an attempt at revascularization) is advised. If there is severe foot necrosis, above-ankle amputation is frequently advised. The spontaneous separation of the viable tissue from the nonviable tissue is known as autoamputation. However, a case series of patients with diabetes-related dry gangrene reported that only 1 of 12 patients experienced autoamputation; the remaining 11 required surgical amputation. It is advised to think about transmetatarsal forefoot amputation rather than multiple toe amputations if more than two digital ray amputations are needed to cure necrosis. Multiple toe amputations can negatively alter pressure distributions and lead to increasing pressure injuries **[17-18]**.

Animal Models:

1) Arachidonic Acid-Induced Hind Limb Gangrene: Rat Model of Peripheral Vascular Disease

Principle: Arachidonic acid, which directly causes platelet aggregation and denudation of the endothelium, has been demonstrated to induce cerebrovascular injury in rats. On the basis of this information we attempted to establish a new model of gangrenous peripheral vascular disease by injecting arachidonic acid into the femoral artery.

Procedure:

Gangrenous Peripheral Vascular Disease Model

Sodium pentobarbital (40 mg/kg, i.p.) was used to anaesthetize the rats before the right femoral artery was exposed and dissected free of all fat and connective tissue. After injecting physiological saline (100 μl) or sodium arachidonate (100 μl, 20 mg/ml in saline; sham operation group) into the artery, the bleeding was stopped with an adhesive agent (Alonalpha-A, Sankyo, Japan), and the wound was then stitched up. According to the modified criteria of Ashida et al., the severity of the hind limb gangrene was evaluated 1 and 10 days after the arachidonic acid injection and scored on a scale of 0 to 7. according to the modified criteria of Ashida *et al.:* 0, normal; 1, gangrene limited to the claws; 2, gangrene limited to the toes; 3, gangrene extended to the sole; 4, gangrene extended to less than half of the ins-tep; 5, gangrene extended to less than two-thirds of the instep; 6, gangrene extended to more than two-thirds of the instep; 7, gangrene extended to the leg.

Histopathological Examination:

Each wounded hind limb was fixed with 10 percent (v/v) formalin, decalcified, and embedded in paraffin either two hours or one day following the arachidonic acid injection. Haematoxylin and eosin was used to stain paw sections taken from various locations.

Platelet Aggregation in Rat Whole Blood: A whole-blood aggregometer (model 560, Chrono-Log, USA) was used to measure platelet aggregation 1 and 10 days after an arachidonic acid injection. The rats were quickly put to sleep with ether anaesthesia before blood was taken from the abdominal aorta into a syringe containing heparin (10 U/ml), which was then added to 490 l of physiological saline in an aggregometer cuvette that was kept at 37 °C. The blood sample was then supplemented with U-46619 (400 nM final concentration) or collagen (250 ng/ml final concentration). Within 7 minutes after administering collagen or U-46619, the results are expressed as the maximal impedance between the two electrodes.

The Number of Platelets, White Blood Cells and Red Blood:

The rats were given ether anesthesia on the first and tenth days after receiving an arachidonic acid injection, and blood was collected from the tail artery into a heparinized syringe. Using a particle counter, the numbers of platelets, white blood cells, and red blood cells were promptly counted (PC- 608, Erma, Japan).

Effect of Thrombocytopenia on Arachidonic Acid Induced Hind Limb Gangrene:

Rats were administered 1 ml of rabbit anti-rat platelet anti-serum intravenously to cause thrombocytopenia. Arachidonic acid was injected to cause hind limb gangrene after 24 hours, and the degree of the gangrene's severity was determined as previously said. The antiserum (1 ml/rat, s.c.) was also administered to the rats 3, 5, and 7 days following the arachidonic acid injection to sustain the thrombocytopenia throughout the trial. After blood was extracted from the tail artery into a heparinized syringe, a particle counter was used to count the amount of platelets (PC-608, Erma, Japan).

Effects of Aspirin and Ticlopidine on Arachidonic Acid-Induced Hind Limb Gangrene:

Two hours before administering the arachidonic acid injection, aspirin (100 mg/kg) and ticlopidine (300 mg/kg) were administered orally. Then, these medications were taken orally for nine

consecutive days. Ten days following the arachidonic acid injection, the degree of hind limb gangrene was estimated and graded as previously mentioned.

2) A Reliable Mouse Model of Hindlimb Gangrene:

Principle: Peripheral artery disease of the lower limbs is a chronically progressive disorder characterized by the presence of occlusive lesions in the medium and large arteries that result in symptoms secondary to insufficient blood flow to the lower extremities. A wide range of symptoms, including severe limb ischemia, characterize Peripheral artery disease. The most severe form of Peripheral artery disease, critical limb ischemia (CLI) caused by atherosclerosis, is the main factor in ischemic rest discomfort, non-healing ulcers, and gangrene or tissue loss. On the basis of this information animal model of gangrenous peripheral artery disease was established by Femoral artery ligation. **METHODS:**

Femoral artery ligation (FAL):

An ischemic condition known as PAD/CLI is typically brought on by decreased tissue perfusion as a result of blocked or partially blocked arteries. FAL results in a more gradual progression of necrosis that starts at the toes and moves up the extremity at a rate that depends on the level of ischemia, compared to other models of tissue ischemia caused, for instance, by ligation of the coronary or cerebral arteries. This provides a more controllable model of gangrenous spread. The simplicity of access to the femoral artery and low mortality rate of this type are additional benefi **[19]**.

The most popular laboratory animal is the mouse, and the three strains that are employed the most frequently are BALB/c, C57BL/6, and FVB mice. We put these three mouse strains to the test by using FAL to cause hindlimb gangrene with or without the addition of L-NAME, an eNOS inhibitor. L-NAME is known to induce ischemia and subsequently necrosis in mouse hindlimbs after FAL by inhibiting nitric oxide (NO) generation and promoting vasoconstriction.

The University of Miami Miller School of Medicine Institutional Animal Care and Use Committee gave its approval to all animal research. The steps we took to cause ischemia in the mouse hindlimbs of BALB/c, C57BL/6, and FVB are outlined below. Male adult mice aged 8 to 10 weeks were intraperitoneally given 100 mg/kg of ketamine and 10 mg/kg of xylazine to induce anesthesia. Depilatory cream was used to stretch, secure, and

eliminate hair from the hindlimbs. A one-centimeter longitudinal skin incision was made from the medial thigh towards the knee while the left hindlimb was being seen under microscopic guidance at 10x or 20x magnification. It was necessary to remove subcutaneous tissue in order to see the femoral artery beneath. The neurovascular bundle was made visible by puncturing the membranous femoral sheath. Dissection and separation of the femoral artery and vein from the femoral nerve were performed proximally beneath the inguinal ligament and distally at the sapheno-popliteal bifurcation, roughly where the external iliac arteries originate. The intermediate length between the sutures was cut with a single incision, and both the artery and vein were ligated distally to these two places with 7-0 silk sutures. A single dosage of sustained-release buprenorphine (ZooPharm, 1.0 mg/kg) was subcutaneously administered after the skin incision was stitched together with 7-0 silk suture. The animal was covered in a heating pad and given a rehabilitation cage to rest in.

Combined FAL and L-NAME Treatment:

Due to genetic predisposition of the BALB/c strain, which includes abnormally developed collateral arteries and defective expression of micro-RNAs that control cell cycle, FAL is well known to preferentially induce necrosis in BALB/c mice but not C57BL/6 mice. As a result, we employed FAL alone to cause necrosis in BALB/c mice and FAL and L-NAME combinations in FVB and C57BL/6 strains to cause gangrene in these stronger strains (see below in the Results section). L-NAME 40mg/kg was progressively injected intraperitoneally into C57BL/6 and FVB mice two hours before surgery as well as on the postoperative days one through three following FAL **[20-21]**.

Ischemia Scoring System:

On PODs 3, 7, and 14, the degree of gangrene in the hindlimb of BALB/c mice was evaluated. Gangrene severity in FVB mice was evaluated on postoperative days (PODs) 1-3 and then every week on days 7, 14, and 21 and 28. The Faber hindlimb appearance score was applied (0 = normal; 1–5 = cyanosis or nail(s) loss, where score represents number of nails affected; 6–10 = partial or complete digit(s) atrophy, where score reflects number of affected digits; 11–12 = partial atrophy or gangrene of forefoot) **[22]**.

Laser Doppler Perfusion Imaging:

An LDI2-HR Laser Doppler Imaging System from Moor Instruments was used to perform Laser Doppler perfusion imaging (LDI) on the ligated and unligated leg before, immediately after, and 7, 14, 21, and 28 days following FAL (Wilmington, DE). Mice were mildly sedated in an induction chamber filled with 1-3 percent isoflurane in 100% oxygen at a flow rate of 2L/minute until they became unresponsive to outside stimuli, as previously described. After that, mice were placed on a heating pad to maintain their temperature between 35°C and 37°C, and pictures of both limbs were examined in the supine and pronated postures using Moor LDI software. The mice were then linked to a nasal cannula with a constant flow of isoflurane/oxygen. The plantar foot was the area of attention because it is responsible for the total perfusion of the legs. The ratio of the ligated to unligated leg was used to express perfusion in order to take into account variations in ambient light, temperature, and arterial pressure. Amputees received a score of 0.

DiI Perfusion:

Traditional CLI mice models' inability to clearly see the regenerated vascular architecture is one of their drawbacks. Micro-computed tomography and micro-angiography techniques are pricy, technically difficult, and not accessible to many laboratories. Lipophilic carbocyanine dye DiI perfusion is a quick and affordable adaption of a vascular staining approach for precise imaging of the mouse hindlimb vasculature. DiI perfusion was carried out by the authors as previously mentioned. At a fraction of the cost, effort, and technological requirements, the procedure offers a resolution that is equal to or better than other methods. The entire foot was peeled after staining, separated from the hindlimb using binder clips, and crushed between two glass microslides for proper imaging with the Zeiss LSM 510 confocal laser scanning microscope. Using Image J high-performance imaging software, Z series from confocal imaging were converted into 3D pictures and evaluated (version k 1.45). For each field of view, vessel volume data were produced by employing a consistent region of interest. The mean vascular density (volume of total DiI stained vessels) for each treatment was assessed using three fields of view from four different mice **[23]**.

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