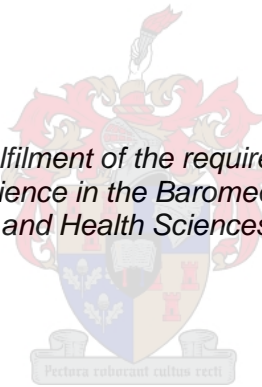


# **A retrospective review of Diabetic Foot Ulcers treated with Hyperbaric Oxygen Therapy in the Kingdom of Bahrain.**

by  
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*Thesis presented in fulfilment of the requirements of the degree of  
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## Declaration

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## Abstract

**Background:** Diabetic foot ulcer (DFU) is a common complication of uncontrolled diabetes mellitus (DM). Ulcers can be neuropathic, ischemic or neuro-ischemic. Its impact on the patient can be dramatic and can lead to amputation and loss of limb function. Hyperbaric oxygen therapy (HBOT) is used as an adjunct treatment to hasten the healing process or limit the extent of the damage caused by ischemia or necrotizing fasciitis. Since patients respond differently to HBOT, this study was carried out to identify factors that influence their response to the treatment after appropriate initial selection.

**Methods:** We performed a case-control study of all patients with DFU treated in the hyperbaric and wound care unit at the King Hamad University Hospital between January 2013 and December 2018. Patients' data were obtained upon patient hospital visit from clinical records. Various baseline factors were compared between patients with and without adequate HBOT treatment responses.

**Results:** A total of 123 patients (cases n= 75 and controls n=48) were included in the study. There was no significant difference in age, gender, duration of DM, weight and body mass index between the groups ( $p>0.05$ ). Cases group ( $11.9 \pm 4.9$ ) had higher white blood cell count than the control group ( $10.1 \pm 3.7$ ) ( $p=0.038$ ). Haemoglobin level was significantly lower in the cases ( $11.4 \pm 1.7$  mg/dl) compared to control group ( $12.3 \pm 1.7$  mg/dl) ( $p=0.009$ ). The number of patients who had new breakdown (19% cases and 4% control) and had smell wounds (45% cases and 21% control) were significantly higher in cases group compared to the control ( $p<0.03$ ).

**Conclusion:** Inflammation and haemoglobin status are major factors influencing wound healing in diabetic patients with ulcer subjected to HBOT.

## Opsomming

**Agtergrond:** Diabeetvoetulkusse is 'n algemene komplikasie van onbeheerde diabetes mellitus (DM). Ulkusse kan neuropaties, isemies of neuro-isemies van aard wees. Die impak daarvan op die pasiënt kan dramaties wees en kan lei tot amputasie en verlies van ledemaatfunksie. Hiperbariese suurstofterapie (HST) word gebruik as aanvullende behandeling om die genesingsproses te versnel of om die omvang van die skade wat veroorsaak word deur isemie of nekrotiserende fasciitis te beperk. Aangesien pasiënte verskillend reageer op HST, word hierdie studie uitgevoer om faktore te identifiseer wat hul reaksie op behandeling beïnvloed nadat hulle toepaslik geselekteer was

**Metodes:** Ons het 'n geval-kontrolestudie uitgevoer van alle pasiënte met diabeetvoetulkusse wat tussen Januarie 2013 en Desember 2018 in die hiperbariese en wondversorgingseenheid by die Koning Hamed Universiteits-Hospitaal behandel is. Die data van die pasiënte is verkry uit die kliniese notas van die hospitaal. Verskeie basislynfaktore is vergelyk tussen pasiënte met en sonder voldoende HST-behandelingsreaksies.

**Resultate:** Altesaam 123 pasiënte (75 gevalle en 48 kontrole) is by die studie ingesluit. Daar was geen beduidende verskil in ouderdom, geslag, duurt van DM, gewig en liggaamsmassa-indeks tussen die groepe nie ( $p > 0,05$ ). Die gevalle ( $11,9 \pm 4,9$ ) het 'n hoër aantal witbloedselle gehad as die kontrole ( $10,1 \pm 3,7$ ) ( $p = 0,038$ ). Die hemoglobienvlak was aansienlik laer in die gevalle ( $11,4 \pm 1,7$  mg/dl) in vergelyking met die kontrolegroep ( $12,3 \pm 1,7$  mg/dl) ( $p = 0,009$ ). Die aantal pasiënte wat nuwe wondafbraak gehad het (19% gevalle en 4% kontrole) en onwelriekende wonder gehad het (45% gevalle en 21% kontrole), was aansienlik hoër in die gevallegroep as die kontrolegroep ( $p < 0,03$ ).

**Gevolgtrekking:** Inflammasie en hemoglobienstatus is die belangrikste faktore wat wondgenesing beïnvloed by diabetiese pasiënte met ulkusse wat HST ontvang.

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# Chapter 1: Introduction and literature review

## 1.0 Introduction

In this chapter, the literature pertaining to Hyperbaric Oxygen Therapy (HBOT) for Diabetic Foot Ulcer (DFU) will be reviewed. The chapter will firstly describe the magnitude of diabetic disease experienced in the world, and particularly in the Middle East. The review will then discuss some complications associated with diabetes and focus on DFU as common complication. An overview of the management of DFU, with a focus on HBOT will set the stage for the context of this study and the chapter will conclude with an overview of different testing modalities that are used in selecting patients with DFU for HBOT.

## 1.1 Diabetes Mellitus and its prevalence

Diabetes Mellitus (DM) is the commonest non-communicable disease, affecting a large proportion of the world population (1). According to the latest statistics of the International Diabetes Federation (IDF) in 2017, the top five countries for the number of people with DM in the Middle East and North Africa are Egypt, Pakistan, the Islamic republic of Iran, Saudi Arabia and Sudan, respectively (2). In Bahrain, the prevalence is high and is rising over time. Musaiger found that the prevalence of DM in Bahraini people was 0.8% in 1980, and in 1982, it was 10.2%(3). In 1996, Zurba and Al-Garf reported that among the Bahraini population the prevalence was 25.5% in men (4). In another study, Al-Zurba found a 25.5% prevalence of Type two Diabetes Mellitus (T2DM) among Bahraini residents who are 20 years and older (5). Aloia and Jassim reported that T2DM among men in Bahrain had increased to a prevalence of 38.30%(6).

DM is a heterogeneous metabolic disorder characterized by the presence of hyperglycemia due to impairment of insulin secretion, defective insulin action or both (7). Broadly, it is classified into type one and type two. However, DM could be secondary to some genetic mutations, exocrine disease of the pancreas or drug exposure. The diagnostic criteria of DM are based on both blood sampling and laboratory methods (8). The diagnosis is made if any of the following criteria are met: A fasting plasma glucose level of 7.0 mmol/L, glycated hemoglobin equal to or more than 6.5%, 2-hour plasma glucose value of  $\geq 11.1$  mmol/L in a 75g oral glucose tolerance test or random plasma glucose  $\geq 11.1$  mmol/L.

## 1.2 Diabetes complications

The chronic state of hyperglycemia in diabetics is associated with complications classified as macrovascular and microvascular, resulting in high morbidity and mortality. Macrovascular complications of diabetes include coronary heart disease, stroke and peripheral vascular disease. Microvascular complications, such as end-stage renal disease, retinopathy and neuropathy, along with lower-extremity amputations, are responsible for much of the burden associated with diabetes (9). Also, cancers, ageing-related outcomes (e.g. dementia), infections and liver disease are all linked conditions (9). Diabetic foot ulcer is another well-known pathological condition associated with diabetes.

## 1.3 Diabetic Foot Ulcer (DFU)

### 1.3.1 Definition and Incidence of DFU

DFU is a full thickness skin injury, necrosis or gangrene that usually occur on the soles of the feet, as a result of peripheral neuropathy or peripheral arterial disease in DM patients (10). Diabetic gangrene is a tissue death caused by occlusion of blood vessels (ischemic necrosis) due to micro-emboli which are caused by peripheral vascular disease as a chronic complication of diabetes.

Worldwide, the incidence of DFU continues to increase (11). Around one in four people with diabetes will develop a DFU in their lifetime (12). It seems that the prevalence of DFU is not accurately known, but is estimated at 4-27% of DM sufferers worldwide (13).

The annual incidence of DFU or necrosis in diabetic patients is known to be about 2% to 5% and the lifetime risk ranges from 15% to 20% (14) (15). The high incidence of DFU complications increases the burden for both patients and their caregivers. The huge economic burden concerns governments in terms of cost of treatment, management of complications, disability and loss of productivity (2).

The type of diabetes is one of the strongest predictors of DFU occurrence. Those diabetic patients who had T2DM mellitus were 2.58 times more likely to develop DFU than those who had T1DM (16). A possible explanation for this would be that in T2DM patients there are other related complications of the disease, such as mechanical deformities in the bony architecture of the foot, peripheral neuropathy, and atherosclerotic changes in peripheral arteries. As a result, the patient may have less tissue epithelialisation, consumption of

oxygen, nutrient transportation, and cell detoxification resulting in ulceration in the extremities (16).

### **1.3.2 Pathophysiology of DFU**

The pathophysiology of DFU is comprised of a number of mechanisms, including neuropathy, vasculopathy and infection:

#### 1.3.2.1 Diabetic Neuropathy

Diabetic neuropathy is the most common cause of diabetic lower extremity ulcers and results in sensory, motor, and autonomic nerve dysfunction (17). With proper screening, approximately 75% of diabetic patients undergoing foot and ankle surgery will be found to have neuropathy (18). Peripheral neuropathy is commonly associated with skin breakdown and neuropathic fractures because of the inability to sense the trauma or an injury. The risk of developing a first DFU has been shown to be 7 times higher in those with moderate or severe sensory loss compared to patients with preservation of sensation (19). Most guidelines recommend the 10g monofilament for neuropathy assessment in people with diabetes and the inability to sense a 10g pressure is the current consensus definition of loss of protective sensation (20).

Diabetic neuropathy can be divided into sensory, motor and autonomic peripheral neuropathy. Evidence for sensory neuropathy includes a reduction or loss of vibration sense (pallhyphaesthesia) and superficial sensitivity (pressure, touch) as well as subjective paraesthesia (21). As the sensation of pain is substantially decreased, the risk for trauma is significantly higher (21). Motoric neuropathy presents as an atrophy of small foot muscles resulting in toe clawing. Above all, loss of Achilles tendon reflex is an early sign of motor neuropathy (22). The combination of both will lead to an unequal pressure distribution and insecure gait. Moreover, hyperkeratosis develops due to elevated plantar pressure load.

Autonomic neuropathy leads to vasomotor dysfunction resulting in arteriovenous shunts of subcutaneous vascular network and the secretion of sweat becomes less (23). Dysfunctional sweating results in dry skin and reduced protective skin function and thus increased risk of injury (21). Moreover, as a result of autonomic neuropathy, medial arterial sclerosis, Charcot's foot (diabetic osteoarthropathy), neuropathic edema, as well as alterations of skin thickness arise (23).

### 1.3.2.2 Peripheral Arterial Disease

Peripheral Arterial Disease (PAD) is a major arterial disease caused by atherosclerosis (24). Diabetes is known as one of the most important risk factors of PAD. Diabetes is associated with a two- to fourfold increase in PAD incidence compared to non-diabetic individuals (25). PAD is an important risk factor for impaired wound healing and lower extremity amputation (26). Even minor injuries accompanied by infection increase the demand for blood supply (including nutrients, oxygen and immune system components) in the foot and an insufficient blood supply results in DFU, commonly leading to limb amputation (27). PAD also inhibits healing by disrupting the processes needed for re-epithelialization (28). In consequence, the American Diabetes Association recommends through consensus that the ankle-brachial index should be performed as a measure of detection in all diabetic individuals >50 years of age or those who have suffered from the disease for more than ten years (29).

### 1.3.2.3 Diabetic Foot Infection

Infection of diabetic foot can represent a dangerous complication of once it involves deeper soft and bone tissues (cellulitis and osteomyelitis) increasing the risk of amputation (30). The infection starts once the skin continuity breaks and opens a door for the bacteria to grow. Although most of the infections remain superficial, such as simple cellulitis, around 25% will spread from the epidermal layer to deeper regions, including subcutaneous tissues and bones, resulting in complications such as necrotic fasciitis, septic arthritis and osteomyelitis (31).

The diagnosis of foot ulcers in diabetics is made by recognizing the presence of pus secretion from infected wounds and certain physical factors, including erythema, tenderness, edema and pain (32). DFU mostly appears to be polymicrobial in nature (33). Both gram-positive (for example *S.aureus*, *E.fecalis*) and gram-negative (*P.aeruginosa*, *E. coli*, *Klebsiella species*, *Proteus species*, etc.) are involved in DFU. The prevalence of these infections in DFUs have been reported to range between 25–60% (34) (35). These different organisms combine together and form micro-communities within a biofilm, which is a matrix of extracellular polymeric substances (33). The formation of biofilm causes many infections to become chronic in nature. The biofilm formation is a resistance mechanism utilised by bacteria against the host immune system or antibiotics (36).

### **1.3.2.4 Classification of DFU**

Edmonds divides DFUs into 2 groups, namely neuropathic and neuro-ischemic (37). Since there is no vascular element in a neuropathic ulcer, the foot is warm, perfusion is good with a palpable pulse, but the perspiration is reduced, and the skin is dry and cracked. On the other hand, feet of neuro-ischemic ulcer patients are cold with no palpable pulse, thin skin without hair and the patient might give a history of intermittent claudication and rest pain may be present.

Apart from the groups described above, a number of classification systems for DFU is known today, such as the Wagner classification (38), the University of Texas wound classification system (UT) (39), and PEDIS (considering Perfusion (ischaemia), Extent (area), Depth, Infection, and Sensation (neuropathy)) (40). Wagner's classification is widely used and describes the extent of the ulcer, but does not describe the state of ischemia (41). Infection is divided into mild infection (superficial, inner and limited in size), moderate (deeper and wider), severe (accompanied by systemic signs or metabolic disorders) (31). Criteria for the diagnosis of infection in DFU include swelling, induration, erythema around the lesion, local pain, palpable local warmth and presence of pus, where two of these criteria are enough for making the diagnosis (42).

The UT system grades ulcers based on depth, which divides patients who have clean, infected and ischemic ulcers and those who have both infection and ischaemia (43). As a result, it has been shown that it predicts major amputation and wound healing (41). The PEDIS system was designed by the International Working Group of the Diabetic Foot (44). It differs from the UT in being designed specifically for prospective research.

The International Working Group on the Diabetic Foot (IWGDF) review found a large number of proposed classification and scoring systems for DFUs, which suggests that none is ideal for routine use in populations worldwide (45). This reflects the different purposes for using a particular classification and scoring system, including communication among health professionals (independent of the level of clinical care), for purposes of clinical prognostication and guidance of treatment, or for clinical audit of outcomes across units and populations (45).

#### **1.3.2.5 Management of DFU**

The main goal in the management of DFU is the closure of the wound (46). Treatment of DFU varies depending on the severity of the ulcer and the presence or absence of ischemia. The basis of DFU therapy is debridement, reducing the pressure on the area of the injury

(“offloading”), managing the infection by providing adequate antibiotics (if required) and ulcer treatment using wound dressing (47) (48).

The Wound Bed Preparation Paradigm provides a structured approach to wound healing (48). This holistic approach addresses the wound in three main aspects; treating the cause, addressing patient centered concerns and local wound management (49). With regards to DFU, an accurate diagnosis of the cause of the ulcer should be established. An acronym VIPs is used to identify the cause of DFU (49). The etiology of DFU considered in the acronym is considered as either vascular, infectious, or neuropathic (or combinations thereof). Early recognition and appropriate treatment of the high-risk foot would save limbs and improve patient quality of life. Infection is defined as critical colonization if superficial, or deep and surrounding wound infection based on the acronyms of NERDS (Non-healing wound, Exudate, Redness, Debris, Smell) and STONEES (Size increasing, Temperature, Os- to bone, New breakdown, Exudate, Erythema, Smell)(49). Patients with DM should be screened for these factors in a systematic manner whenever they are considered for HBOT.

## **1.4 Hyperbaric oxygen therapy**

Hyperbaric oxygen therapy (HBOT) is a treatment designed to increase the body oxygen by administering 100% oxygen at higher than atmospheric pressure. Henry’s Law dictates that the relationship between the volume of gas dissolved in a liquid or tissue and the partial pressure of that gas is proportional(50). Therefore, increasing atmospheric pressure will cause more oxygen to dissolve in the plasma, thereby maximizing tissue oxygenation (51).

### **1.4.1 History of HBOT**

The use of oxygen at elevated pressures was first proposed for the treatment of decompression injury (52). Later, Churchill-Davidson et al described the use of elevated pressures and oxygen to potentiate radiotherapy in cancer patients in 1955 (53). In 1956, Boerema et al published a paper on the clinical use of hyperbaric oxygen to extend the duration of circulatory arrest during cardiac surgery (54). These publications were followed by reports of the clinical response to HBOT in patients suffering from Clostridial infections (54) and those poisoned by carbon monoxide (55). Within less than a decade, a diversity of medical disciplines were supporting the use of high oxygen tensions at pressure for the treatment of various disease states (56), although controversy regarding its use also existed (57).

### 1.4.2 Mechanism of Action and HBOT Uses

HBOT exerts its therapeutic effect by four mechanisms: mechanical effects, bacteriostatic effects, hyperoxygenation, and finally, the correction of hypoxia (58). Oxygen plays an important role in all cellular processes during wound healing, including cell metabolism, proliferation, and revascularization (56). Oxygen is also essential for increased antimicrobial activity, growth factor signal transduction, and collagen synthesis (56).

Hyperbaric therapy has been used in the treatment of non-healing wound repair in chronic diabetic ulcers and delayed post-radiation tissue injuries. These disorders share many common elements, including chronic inflammation, reduced oxygen supply, stromal cell depletion, and fibrosis (59). Hyperbaric therapy has been shown to promote angiogenesis, enhance fibroblast activity, augment formation of granulation tissue, reduce edema, and improve leukocyte function. Vasculogenesis is enhanced by HBOT-induced mobilization of stem cells from bone marrow (60). Neovascularization occurs by regional angiogenic stimuli, which influence the efficiency of new blood vessel growth by local endothelial cells (termed angiogenesis) and they stimulate the recruitment and differentiation of circulating stem/progenitor cells (SPCs) to form vessels *de novo* in a process termed vasculogenesis (61) (62).

The activity of bone marrow endothelial nitric oxide synthase, which is required for SPCs mobilization, is diminished in DM (63). HBOT mobilizes SPCs in patients previously exposed to radiation and in diabetics (60). Moreover, HBOT mediated oxidative stress at sites of neovascularization will stimulate SPCs growth factor production by augmenting the synthesis and stabilization of hypoxia inducible factors (64). Extracellular matrix formation is closely linked to neovascularization and it is another oxygen-dependent process (65).

HBOT was shown to increase the synthesis of vascular endothelial growth factor and it is the most specific growth factor for neovascularization (66). HBOT also stimulates synthesis of basic fibroblast growth factor (bFGF) and transforming growth factor  $\beta$ 1 by human dermal fibroblasts (67), angiopoietin-2 by human umbilical vein endothelial cells (68), bFGF and hepatocyte growth factor in ischemic limbs and it up-regulates platelet derived growth factor (PDGF) receptor in wounds (69).

HBOT has been demonstrated to have bacteriostatic and bactericidal effects. One study has demonstrated both pressure and hyperoxia to be important in the interaction between bacteria and neutrophil-like cells (70). Antimicrobial activity is potentially detrimental to



wound healing through development of a pro-inflammatory environment, but enhanced apoptosis can resolve the inflammation and support the progression of wound healing (70).

There is growing evidence to support the promotion of bone repair with HBOT in chronic wounds with osteomyelitis. Osteoblast stimulation, anti-osteoclastic effects, and bone regeneration have been demonstrated using intermittent oxygen supplementation, providing mechanistic evidence for the adjunctive use of HBOT (71). Mader and Niinikoski demonstrated that the decreased oxygen tensions typically associated with bony infections could be returned to normal or above normal levels while breathing 100% oxygen in a hyperbaric chamber (72). Neutrophils require tissue oxygen tensions of 30-40 mmHg to destroy bacteria by oxidative killing mechanisms (73). HBOT has been proven effective as adjunctive therapy in animal models of chronic *S. aureus* and *Pseudomonas aeruginosa* osteomyelitis (74). Moreover, the transport of aminoglycoside (gentamicin, tobramycin, amikacin) across the bacterial cell wall is oxygen-dependent and is inhibited in conditions of a hypoxic environment when the tissue oxygen tensions are below 20 to 30 mmHg. Therefore, HBOT therapy may enhance transport and augment the antibiotic efficacy (75). This synergistic effect has also been shown for the cephalosporin class of antibiotics, where the combination of cefazolin and HBOT therapy produced a 100-fold greater reduction in bacterial counts than either antibiotic or HBOT therapies alone (76).

### **1.4.3 Patient Selection for HBOT**

Patients with chronic wounds selected for HBOT usually have a history of non-responsiveness to conventional treatments, including antibiotics and topical dressings, and failed debridement.

Patient selection for HBOT can be assisted by noninvasive transcutaneous oxygen monitoring (TCOM) (56). HBOT is inappropriate when it is provided to patients who could heal without it, or when it is provided to patients who would most likely not benefit at all, or when an excessive number of treatments is provided to achieve the desired benefit (77). Most hyperbaric units select their patients based on TCOM measurements.

## **1.5 Trans-Cutaneous Oxygen Measurement**

The appearance of commercial transcutaneous pO<sub>2</sub> monitors in 1977, and transcutaneous pCO<sub>2</sub> monitors in 1978, represented a timely fusion of physiological understanding and

technical innovation (78). The first 'combined' transcutaneous pO<sub>2</sub> and pCO<sub>2</sub> monitoring system was described in 1979 (79).

Non-invasive TCOM can identify patients unlikely to heal spontaneously and most likely to benefit from HBOT. The IWGDF systematic review shows the most useful tests for predicting healing in an ulcerated patient were skin perfusion pressure ( $\geq 40$  mmHg), toe pressure ( $\geq 30$  mmHg) and TCOM ( $\geq 25$  mmHg) (80).

Healing of an ulcer in a patient with peripheral arterial disease is related to the interplay of the severity of the perfusion deficit with other characteristics such as amount of tissue loss, presence of infection, mechanical load on the ulcer and other comorbidities (81). Patients with a toe pressure  $< 30$  mmHg or a TCOM  $< 25$  mmHg carry poor chance of healing and IWGDF suggest considering imaging and revascularization in these patients (82). However, it should be noted that peripheral arterial disease is not the only cause of reduced perfusion in a lower extremity, since edema and infection may also result in poorer tissue oxygenation, and these should all be treated accordingly (83) (84).

## **1.6 Studies regarding HBOT in the management of DFU**

Many studies of the management of chronic wounds that included HBOT as component in the treatment have been undertaken during the past 45 years, including studies involving different types of lower limb ulcers.

Kranke et al performed a Cochrane review of HBOT for chronic wounds, identifying 27 potentially eligible trials, but excluded 17 based on quality (85). They found seven trials comparing HBOT as a treatment for diabetic ulcers with controls (68, 86-91), one study comparing HBOT with extracorporeal shock-wave therapy (92) and one study on HBOT for venous ulcers (93). They concluded that there was some evidence that HBOT used adjunctively for diabetic wounds results in significant short-term improvement of wound healing by 6 weeks (85).

Despite these findings, some studies found the opposite. For instance, a publication in February 2013 called "Lack of effectiveness of hyperbaric oxygen therapy for the treatment of diabetic foot ulcer and the prevention of amputation" by D.J. Margolis et al (94) contradicts the previous studies. However, several points of criticism against the study have been reported in a number of recent commentaries (95-97). These included the design of the study, interpretation of the findings and the analysis methods of the study. These

commentaries stressed again that proper patient selection is a main predictor of the effectiveness of the treatment.

## **1.7 Gaps in the literature and further studies needed**

HBOT has been used in the treatment of DFUs for more than 20 years, typically using the protocols described in the Undersea and Hyperbaric Medical Society guidelines (98). However, despite following the recommended protocols for patient selection and follow-up, there are still a number of patients who seem to be poor responders to the treatment. It seems like further differentiation is required in patients who have already been selected for HBOT. No such information is available in the literature, apart from the limited studies alluded to above.

The identification of factors that may predict a poor treatment response is important. If these factors are considered in addition to existing patient selection factors, it could potentially lead to the identification of additional modifiable risk factors that could be incorporated into the management protocol of patients in the unit, or a refined patient selection process could be implemented (in cases where these factors cannot be modified).

## **1.8 Chapter conclusion**

This chapter reviewed the literature pertinent to DFU and how HBOT may directly address specific causative factors. Although protocols for the selection of patients exist, a significant proportion of individuals do not respond to HBOT as expected. Further stratification of patients may identify additional modifiable risk factors (and hence a requirement to include additional treatment regimens) or patients who would not respond to treatment may be identified and the selection of patients may therefore be improved further.

This study aimed to explore these concepts at a busy HBOT unit, and the study methods will be addressed in the next chapter.

In conclusion, the prevalence of DM is increasing in Bahrain and worldwide. Diagnosis of DM is based on fasting blood glucose, glycated haemoglobin or oral glycaemic challenge. Complications are classified as macrovascular and microvascular. Diabetic foot ulcer is one of the complications and continues to increase worldwide causing huge economic burden on the government. Diabetic neuropathy and peripheral arterial disease are the major causes of DFU. Treatment of DFU is based on multidisciplinary approach. Hyperbaric oxygen therapy

is been used to treat DFU and that is for its known effect on angiogenesis, promoting granulation tissues formation, reducing the edema and improving leukocyte function.

## Chapter 2: Study aim and objectives

### 2.1 Study Aim and Objectives

The main aim of this study was to assess potential factors that may influence the outcome of diabetic foot ulcers treated with an appropriate course of HBOT.

The study thesis includes that this information may allow for more detailed and better selection of patients for HBOT and allow for better prediction of their response to therapy.

#### 2.1.1 Primary objectives

The study therefore had the following primary objectives:

1. To identify patients with a poor treatment outcome despite receiving an appropriate course of HBOT for DFU. This included study participants:
  - with DFUs that do not show at least 30% reduction in the wound surface area in four weeks (at the end of 20 sessions of HBOT); or
  - requiring major amputation, including the ankle joint and above, within 3 months following completion of HBOT; or
  - who developed a new DFU on the same foot within 3 months following completion of HBOT
2. To describe the association of different variables with the outcome of HBOT in patients who received treatment for DFU, including
  - Demographic variables, including age and sex;
  - Anthropometric variables, including height, weight and Body Mass Index;
  - Baseline blood test values (Creatinine level, Haemoglobin and Glycosylated Haemoglobin, White Cell Count and C-reactive protein);
  - Information about their diabetes, such as duration of their diabetes since diagnosis and co-morbid diseases the patient had been diagnosed with;
  - Transcutaneous Oxygen Measurement in the wound area, as described in international guidelines (99);
  - Information about ulcer duration, infrared thermometer and pain score;

- Information about ulcers such as cause (vascular, infection, pressure, burn or trauma) (100), site (toe, metatarsal, heel), depth (superficial, deep, to bone), Texas classification (101), NERDS and STONES criteria (102);
- The presence or absence of foot deformities, such as clawing of the toes, hallux limitation, pes equinus, etc.);
- History of vascular surgery in the lower limb and vascular assessment of the foot (presence or absence of pedal pulses, Doppler examination of foot pulses); and
- Sensation assessment of the foot using a 10-g monofilament and the 60-second screening tool (103).

### 2.1.2 Secondary objectives

The secondary objectives of the study were based on the primary objectives and included:

3. to identify predictors of “high risk patients” who may require additional care when receiving HBOT for DFU, and
4. to identify predictors of “high risk patients” who would unlikely benefit from HBOT for DFU and should rather be selected out (patient stratification).

## 2.2 Independent variables

In order to determine whether any baseline information obtained from study participants could predict the outcome of this study (adequate or inadequate response to HBOT), the following variables were assessed in the cases and controls:

<b>Variable</b>	<b>Description</b>	<b>Measurement</b>
Age	Age of the study participant at the time of starting HBOT	years
Sex	The biological sex of the study participant	Classified as male or female
Height	The height of the participant	in centimetres
Weight	The weight of the participant	in kilograms

Body Mass Index	The weight of the participant in kilograms by the square of their height in metres	Index
Duration of diabetes	The duration that the patient had been diagnosed with diabetes (from date of diagnosis to date of first consultation)	Years
Co-morbid diseases	A list of all the co-morbid diseases with which the participant was diagnosed previously	List of diagnoses
Pedal pulses	Whether the pedal pulses are present or absent during palpation as part of the clinical examination upon admission to the unit	Dorsalis pedis pulse and/ or posterior tibialis pulse present or absent
Doppler examination of foot	The Doppler pulse wave of the dorsalis pedis and posterior tibial pulses	Biphasic, triphasic or absent
TCOM on air	The lowest TCOM value measured in close proximity to the wound after breathing room air	Value in mmHg
TCOM on oxygen	The lowest TCOM value measured in close proximity of the wound after breathing 100% via face mask at sea level for 15 minutes.	Value in mmHg
TCOM with chamber challenge	The lowest value of the TCOM after breathing 100% oxygen at a pressure of 200kPa.	Value in mmHg
Vascular surgery	Whether the participant has a history of previous vascular surgery (e.g. angioplasty) related to the lower limb	A positive history was further elucidated by the date and anatomical location
Baseline blood results	The laboratory-determined values of the following: <ul style="list-style-type: none"> <li>• Baseline serum creatinine</li> <li>• Baseline HbA1c</li> <li>• White blood cell count</li> <li>• Hemoglobin level</li> <li>• C-reactive protein level</li> </ul>	The value obtained from the laboratory
Presence or absence of neuropathy	Assessment of sensation using the 60-second screening tool(103) and neuropathy is considered to be present if the participant was unable to detect four or more sensory	Present or absent

	challenges with the 10-g monofilament.	
Presence or absence of foot deformities	Clinical evaluation of the patient to determine whether specific deformities are present, such as clawing of toes, hallux limitation, pes equinus, etc.	Present or absent, with listing the specific deformity
The cause of the wound	Vascular, Infective, Pressure, Trauma or Burn	The main causes for the wound is listed
Pain	The amount of pain the participant experienced upon admission, as measured with a visual analogue scale	Rated from 0 to 10
Ulcer site	The anatomical site of the ulcer	toe, hallux, metatarsal head, other
Ulcer depth	The depth of the ulcer (deepest)	Superficial, deep, or to bone
Ulcer duration	The duration of suffering the ulcer as reported by the participant	weeks
Presence of other surrounding deep tissue injuries	Whether the patient has other deep tissue injuries in the same foot	Present or absent
The stage of the wound	Application of the Texas classification system to the wound	A (0-3) B (0-3) C (0-3) D (0-3)
Osteomyelitis present	Whether osteomyelitis is diagnosed on the foot X-rays performed	Present or absent
Charcot foot	Whether Charcot foot is diagnosed on the foot X-rays performed	Present or absent
Sharp debridements	The number of sharp debridements the patient had received at the time of admission (prior to receiving HBOT)	Numerical count
Surface skin Temperature	The highest temperature in the area of the wound, as measured by the infrared thermometer	Degrees centigrade
Wound infection/ inflammation	NERDS and STONEES criteria were applied	Whether each of the criteria was present or absent



Microbiological culture	Whether any micro-organism was cultured in the wound	Yes/ no and the specific organism being listed
Moisture balance	The moisture balance of the wound in the wound, to indicate whether the wound is dry (moisture was added) or wet (moisture was taken away)	added or taken away
HBOT sessions	The number of HBOT sessions received by the participant	Numerical count
Rate of wound healing	The rate at which the wound was healing, as determined by using a 3-dimensional camera and progress is plotted with each visit.	Percentage of wound reduced at the end of 20 sessions
Treatment outcome	Classification of the individual participant as a "case" or "control" in accordance with the criteria provided above	Case or control

## Chapter 3: Methodology

### 3.1 Chapter Introduction

The previous chapter detailed the aim and objectives of the study. This chapter will provide details about the methodology that was followed to realize the aim and objectives. It will describe the study design, as well as the setting in which the study was performed, so that appropriate extrapolations could be made to similar settings in future. A detailed description of the study participants and how they were selected and the different variables that were measured is provided.

The chapter will also indicate the way in which the data was obtained from study participants and how the data was managed, including the statistical analyses.

The chapter concludes with the important ethical considerations that were pertinent in this study.

### 3.2 Research Design

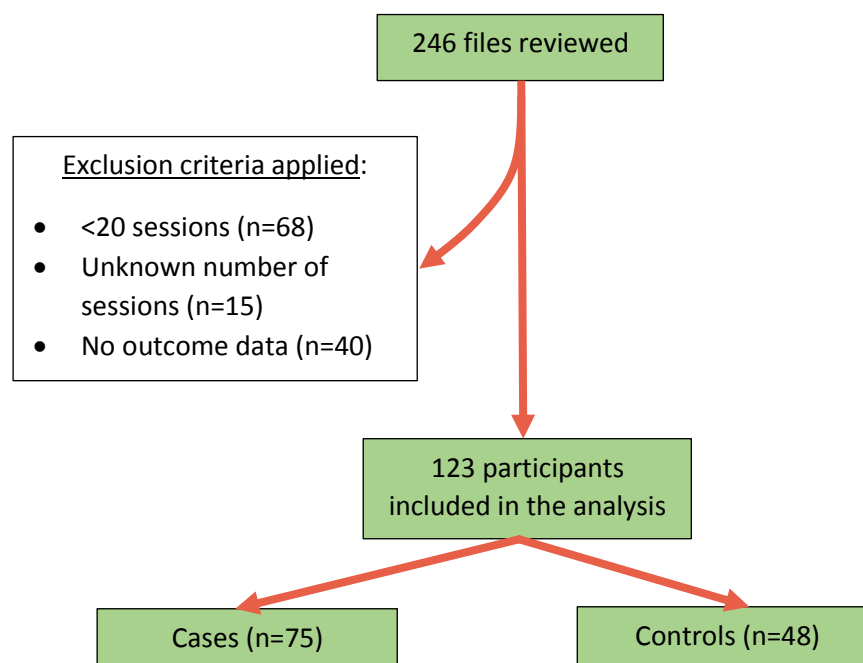
This study employed a retrospective nested case control study to realize the aim and objectives of this study. This design was used as the outcome is already available (healed or not healed), but the exposure of interest is not been studied. Because we were limited in the budget, time and availability of information we opted to use this study.

### 3.3 Study Participants

After excluding all the patients who received HBOT for indications other than DFU during the study period (01 January 2013 to 31 December 2018), a total of 246 patients met the inclusion criteria for the study and were treated for Diabetic Foot Ulcers (DFU) occurring below the level of the malleolus. Their files were reviewed in more detail to determine whether they meet the inclusion and exclusion criteria.

Figure 4.1 provides a flow-diagram indicating the flow of these participants in different phases of the study. A total of 83 combined patients received <20 treatment sessions (n=68), or received an unknown number of treatment sessions (n=15) as part of their treatment course and were thus excluded from the sample in accordance with the inclusion and exclusion criteria of the study. A further 40 patients did not have follow-up information available to enable classification as cases and controls. A total of 123 patients were

therefore included in the study. These individuals contributed a total of 3,579 patient-weeks of follow-up.



**Figure 4.1: Flow diagram indicating the number of participants during different phases of the study.**

All patients who were seen at the King Hamad University Hospital (KHUH) in the Hyperbaric Oxygen Therapy (HBOT) unit were eligible for enrolment in the study if they met all of the following inclusion criteria:

- Admitted to the unit between January 2013 and December 2018;
- Received HBOT for DFU as indication;
- Received the full workup in accordance with the existing unit protocols; and
- Received advanced wound care (that means the patient has been evaluated using the advanced wound evaluation and treatment modalities and been treated using the advanced wound care facility protocols and procedures)

Exclusion criteria that were used in the study were:

- Ulcers above the malleolus;
- Patients who received less than 20 HBO treatment sessions; or
- Patients for whom outcome information was not available in their clinical folders

### 3.4 Outcome variables

Once the full cohort of patients who received HBOT for DFU (as defined in the inclusion and exclusion criteria) were selected, we divided them into cases and controls, based on the following definitions:

Cases: Participants had an “inadequate response” to the HBOT, which was defined as:

- Wounds that did not show at least 30% reduction in the wound surface area at the end of 20 sessions of HBO treatment (104); or
- Participants requiring a major amputation, including the ankle joint, within 3 months following completion of HBOT; or
- Participants who developed DFU on the same foot within 3 months following the course of HBOT

Controls: All participants who did not fulfil the criteria defined above as an “inadequate response” were used as controls in the study.

### 3.5 Study setting

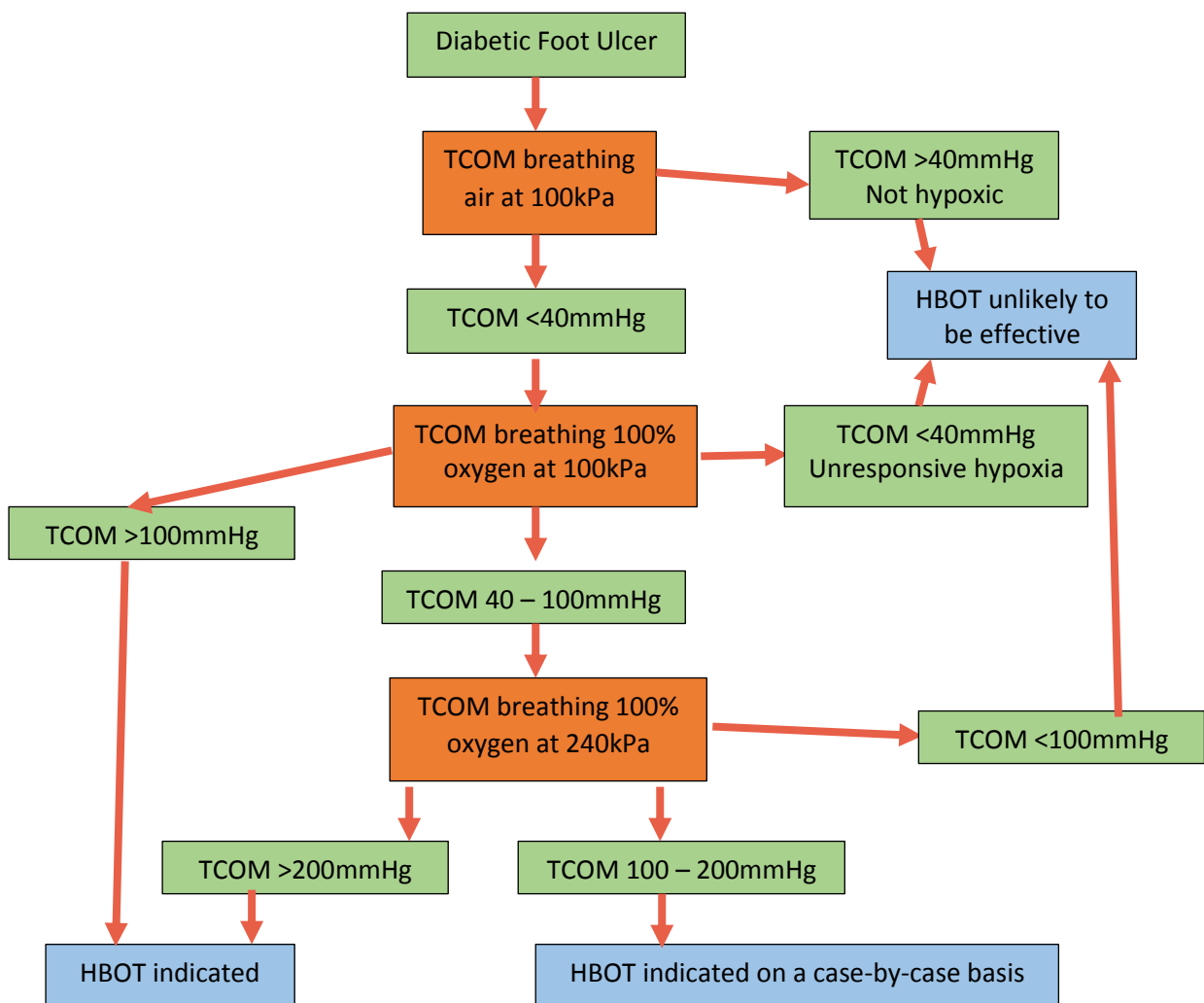
King Hamad University Hospital (KHUH) in the Kingdom of Bahrain represents one of the major tertiary hospitals. It harbours the biggest HBOT unit in the Middle East. The unit was established in 2011 and has two different kinds of hyperbaric chambers, monoplace and multiplace chambers. A monoplace hyperbaric chamber is generally made of acrylic material to permit direct patient observation. Multiplace chambers are typically steel constructions in which more than one patient is pressurized at a time. The multi-place unit at KHUH is certified for pressurization of eight patients per treatment session and it houses 5 monoplace chambers.

The unit conducts approximately 4000 HBOT sessions per year, of which Diabetic Foot Ulcers (DFU) comprises the major proportion of indications for which treatment is provided. The unit is also linked to an advanced wound care facility that receives referrals from all other departments in the hospital and within the Kingdom. It encompasses 9 beds, of which 6 are for ambulatory patients and the rest are designed to receive patients via ambulance. This unit is equipped with advanced devices that help in making accurate diagnoses, such

as Transcutaneous Oxygen Monitoring (TCOM) equipment, Ankle Brachial Pressure Index (ABPI), an 8MHz handheld Doppler and infra-red thermometers.

Patients are referred to the unit from within the hospital, as well as from practitioners outside the hospital. All patients are consulted by a medical practitioner who is qualified in providing HBOT and is supported by the wound care personnel in case of patients referred with wounds. Patients are evaluated in line with international guidelines for wound management (in the wound care centre) and some of them may be selected for adjunctive HBOT if they meet the international criteria and guidelines.

The protocol for selection of patients based on TCOM measurements is shown in Figure 3.1.



**Figure 3.1. Selection of diabetic patients for HBOT based on TCOM values**

The HBOT sessions are provided once daily for patients, five days per week. Standardised unit protocols (based on the international guidelines) are used in the selection and management of patients. This includes standardized unit documentation to capture clinical information.

### **3.6 Data Sources and management**

As part of this process, a study number was assigned for each individual and no personal identifying information was captured. All information required for this study was captured in a single sitting for any one patient, to ensure access to the records are not required again. The patient treatment logs were used as sampling frame.

The clinical records of the patients consisted of the clinical notes (captured by the treating physician), the side-room investigations as captured by the nursing personnel of the unit, the detailed wound assessment information that consists of standard patient information capture sheets for systematic assessment, the laboratory information of all special investigations performed, including X-ray reports and the detailed log sheets of all the HBOT sessions the patient received.

### **3.7 Sample size calculation**

In order to maximize the power of the study to detect a difference between cases and controls, the study aimed to include all individuals (n=246) who were treated at the facility during the study period – provided they meet the inclusion and exclusion criteria. All individuals (n=123) who met the criteria for classification of cases (inadequate response to treatment) were included in the study and the rest of the individuals were all classified as controls.

### **3.8 Quantitative Variables**

Quantitative variables in this study were summarized as means (with standard deviations) or as medians (with interquartile ranges) if the data was not normally distributed. Population estimates were determined by using 95% confidence intervals.

### 3.9 Statistical methods

Data were presented in graphs and tables. Differences between cases and controls were determined using the following statistical methods:

- Numerical variables were compared by using the F-test for comparison of the variances and then using the T-test to compare the mean values in the populations, assuming equal or unequal variances as determined by the F-test. If the data was not normally distributed (as determined by means of normality plots), the Wilcoxon rank-sum test was used.
- Categorical variables were compared using the Chi-squared test of contingency tables. The 2x2 tables were additionally evaluated by calculating the Odds Ratio (with 95% confidence intervals). Whenever the individual cell frequency assumptions were violated, the Fisher's exact test was used. If one of the cells in the 2x2 table was empty, the method for approximation as described by Cornfield was used to allow for statistical analysis (105).
- Percentages and proportions were compared using the Z-test.

All of these statistical procedures were performed using the Stata statistical software package (Stata corp). A significance level of 0.05 was used in all statistical tests.

A binary logistic regression model was developed by inserting all independent variables that were statistically significantly associated with the outcome variable ( $p < 0.05$ ). This was performed using the SPSS statistical software package.

### 3.10 Ethics considerations

The study was approved by the King Hamad University Hospital ethics committee (reference: KHUH/Research/No.227/2018).

The study complied with the international legal and ethical principles as contained in the Declaration of Helsinki (106). The main ethics consideration of concern in this study was the principle of autonomy.

The international guidelines requires that informed consent must be obtained from the patients before participation in research. However, in considering this particular study, it was exceptionally difficult to obtain consent from study participants, since they had all been discharged from the unit and referral to the unit was from a very large geographical area

across the Kingdom of Bahrain and other countries in the Middle East. The investigators therefore submitted a motivation and request for waiver of informed consent. At the same time the particular importance of maintaining absolute confidentiality in this context in order to protect the individuals was stressed.

In order to ensure confidentiality, no identifying personal information was captured during this study. Also, the only persons who had access to the primary data (patient files) were the individuals who treated these patients and thus generated the information. No additional persons had access to the medical information captured in the. The primary investigator captured all the information directly from the patient files into the spreadsheet and the database therefore only contained de-identified information. Only this de-identified information was shared with the study supervisor and statistician.



## Chapter 4: Results

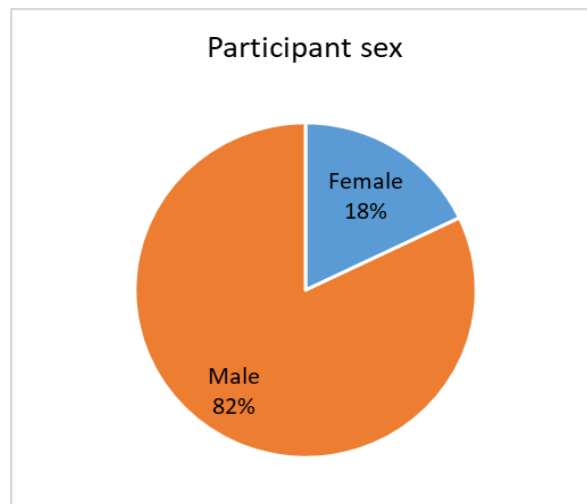
### 4.1 Chapter Introduction

This chapter will first describe the demographic profile and descriptive statistics of the study participants, and then provide the results of the analytical statistical analyses.

### 4.2 Descriptive statistics

#### 4.2.1 Patient characteristics

The sample cohort (n=123) comprised 22 females and 101 males. Figure 4.2 shows the proportion of study participants that were male and female. Males represented majority of the participants with only 18% of participants being female.



**Figure 4.2: Distribution of patient characteristics by sex**

Additional demographic, anthropometric and laboratory data of the study participants are contained in Table 4.1.

<b>Table 4.1. Demographic, anthropometric and laboratory data of all study participants (N=123)</b>			
<b>Variable</b>	<b>N (missing)</b>	<b>mean (SD)</b>	<b>Median (IQR)</b>
Age (years)	123 (0)	58.2 (10.1)	58.1 [51,17 - 64,98]
Height (cm)	79 (44)	171.7 (8.6)	173 [165.5 - 177.7]
Weight (kg)	79 (44)	86.4 (14)	86.3 [77.65 - 94.6]
BMI	79 (44)	29.3 (4.6)	28.4 [26,19 - 33,09]
DM duration (weeks)	118 (5)	18.5 (8.5)	20 [11 - 24,5]
Creatinine level	103 (20)	138.7 (150.5)	96.5 [83,36 - 121,56]
Baseline HbA1c (%)	100 (23)	8.8 (1.7)	8.5 [7,6 - 9,63]
Baseline WBC	102 (21)	11.2 (4.5)	11.4 [7,67 - 13,66]
Baseline haemoglobin	103 (20)	11.8 (1.7)	11.7 [10,6 - 13,1]
Baseline CRP (mg/l)	82 (41)	87.4 (100.1)	45 [16,32 - 150,7]
No of HBOT sessions	123 (0)	27.1 (7)	27 [20 - 30]

Table 4.2 compares the demographic, anthropometric and laboratory results of cases (those with an “inadequate response” to HBOT) and controls (those who responded optimally). No significant difference were found in age, sex, height, weight, or body mass index between cases and controls ( $p>0.05$ ). There were also no significant differences in their creatinine, C-reactive protein and HbA1c levels ( $p>0.05$ ). However, cases had significantly higher white blood cell counts and lower haemoglobin levels compared to controls ( $p<0.05$ ). The haemoglobin levels did not differ between males and females ( $p=0.080$ ).

**Table 4.2: The demographic, anthropometric and laboratory results in the cases and controls**

<b>Variables</b>	<b>Cases (n= 75) Mean <math>\pm</math> SD Median [IQR]</b>	<b>Controls (n=48) Mean <math>\pm</math> SD Median [IQR]</b>	<b>p-value</b>
Participant age (years)	58.4 $\pm$ 10.1	57.8 $\pm$ 10	0.764
Proportion who is male	64 / 75	11 / 48	0.295
Duration of DM	20 [12-22]	20 [10-25]	0.615
Weight (kg)	86.2 $\pm$ 14.4	86.6 $\pm$ 13.7	0.898
Height (cm)	171.9 $\pm$ 7.1	171.6 $\pm$ 10.2	0.873
Body Mass Index (kg/ cm <sup>2</sup> )	28.3 [25.6-32.9]	28.4 [27.1-33.2]	0.542
White blood cell count	11.9 $\pm$ 4.9	10.1 $\pm$ 3.7	0.038
Haemoglobin level	11.4 $\pm$ 1.7	12.3 ( $\pm$ 1.7)	0.009
Creatinine level	100.9 [84.7-124.8]	93 [80.4-117]	0.175
HbA1c	8.4 [7.9-9.8]	8.6 [7.6-9.4]	0.595
C-Reactive Protein mg/L	47.6 [16.2-194]	39.5 [16.5-66.5]	0.232

#### 4.2.2 Outcome variables

Of the 123 study participants, (61.0%, n=75) of the study participants had an “inadequate response” to HBO therapy, yielding 48 controls for comparison. The cases comprised sixty-five individual participants who had a single “inadequate response” item that designated them as cases. Of these individuals, 72% (n=54) did not have 30% reduction in their wound areas, 13.3% (n=10) had a new DFU within 3 months following their treatment and one individual had an amputation above the ankle within 3 months following the treatment. A further 10 study participants had two “inadequate response” items that designated them as cases (six individuals had less than 30% reduction in the size of their wounds and also had an amputation above the ankle, three had less than 30% wound reduction and also

developed a new wound within three months and one individual had a new DFU and amputation within three months following treatment).

### 4.2.3 Predictive variables and analytical statistics

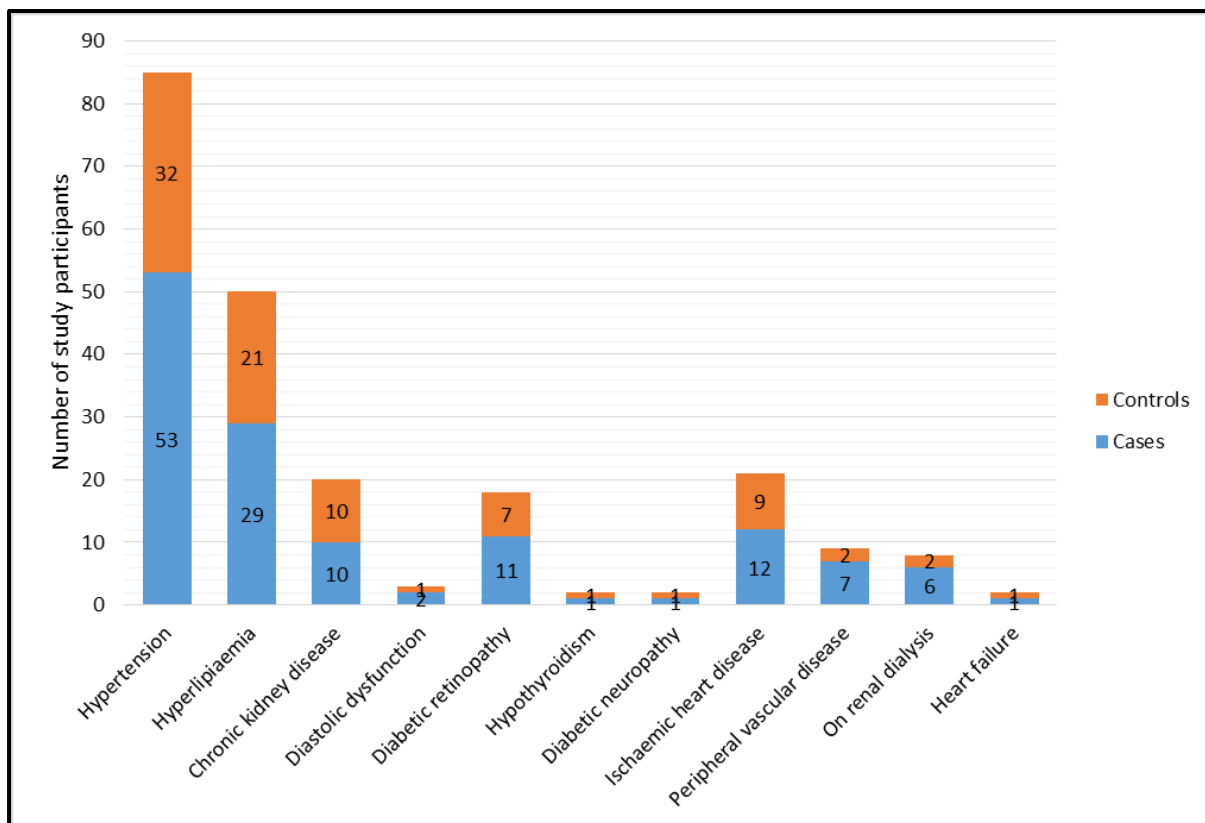
Table 4.3 Shows the results of TCOM measurements on air and after 15 minutes of breathing 100% oxygen via face mask, pain score and ulcer duration, none of which showed significant differences between cases and controls. Conversely, cases had a significantly higher infrared thermometer reading compared to controls ( $p=0.001$ ).

<b>Variables</b>	<b>NOH (n= 75) Mean <math>\pm</math> SD Median [IQR]</b>	<b>OH (n=48) Mean <math>\pm</math> SD Median [IQR]</b>	<b>p-value</b>
Lowest TCOM (mmHg)	39.5 $\pm$ 16.3	39.3 $\pm$ 15	0.944
Lowest TCOM after 15 mins O <sub>2</sub> by face mask (mmHg)	110 [88-148.8]	103.3 [83.5-145.5]	0.685
Pain score	3.5 [2-6]	3 [2-5]	0.924
Ulcer duration (weeks)	2 [1-4]	2 [1-3]	0.340
Infrared thermometer ( $^{\circ}$ C)	5.5 [4-7]	3.5 [2.3-5]	0.001

### 4.2.4 Comorbidities

Only 15% of patients (n=18) had DM with no other comorbid conditions captured in their clinical files. The majority of the patients (n=100 (85%)) had at least one comorbid condition.

Figure 4.3 represents the number of participants presenting with specific comorbidities (with many patients having more than one comorbidity). The majority of the patients (n=85) had hypertension as comorbidity of their DM. Diabetic nephropathy and hypothyroidism were not common comorbidities in this cohort.



**Figure 4.3: The number of patients with comorbidities. Numbers on the bars represent the number of the patients for each comorbidity.**

	Cases	Controls	p-value
Hypertension	72%	67%	0.626
Hyperlipidaemia	39%	44%	0.723
Chronic kidney disease	14%	21%	0.680
Diastolic dysfunction	3%	2%	0.960
Diabetic retinopathy	15%	15%	1.000
Hypothyroidism	1%	2%	(insufficient observations)
Diabetic neuropathy	1%	2%	(insufficient observations)
Ischaemic heart disease	16%	19%	0.857
Peripheral vascular disease	9%	4%	0.817
On renal dialysis	8%	4%	0.848
Heart failure	1%	2%	(insufficient observations)

The causes of the wounds did not differ between cases and controls ( $p > 0.05$ ). The details of the causes of the wounds of study participants are contained in Table 4.5.. Likewise, the sites of their diabetic foot ulcers and the depth of their ulcers did not show statistically significant differences (see Table 4.6).

<b>Variables</b>	<b>Cases number (%)</b>	<b>Controls number (%)</b>	<b>p-value</b>
Vascular	18(24)	10(21)	0.848
Infection	37(50)	23(48)	0.915
Pressure	7(9)	7(14)	0.762
Trauma	18(24)	7(15)	0.606
Burn	2(3)	4(8)	0.790

Likewise, the sites of their diabetic foot ulcers and the depth of their ulcers did not show statistically significant differences (see Table 4.6).

<b>Site</b>	<b>Cases number (%)</b>	<b>Controls number (%)</b>	<b>p-value</b>
Toe	44 (59)	28 (37)	0.978
Metatarsal	24 (32)	22 (29)	0.336
Heel	20 (27)	6 (8)	0.472
<b>Depth</b>			
Superficial	8 (11)	13 (17)	0.368
Deep	48 (64)	27 (36)	0.509
Bone	19 (25)	8 (11)	0.624

<b>Table 4.7: Texas classification of wounds, NERDS, STONEES and presence of foot deformity in cases (n=75) and controls (n=48)</b>			
<b>Variables</b>	<b>Cases (n= 75)</b>	<b>Controls (n=48)</b>	<b>p-value</b>
<b>Texas classification</b>			
A0	0	1(2)	0.191
A1	0	0	
B0	0	1(2)	
B1	4 (5)	9 (19)	
B2	27 (36)	19 (40)	
B3	22 (29)	9 (19)	
C0	1 (1)	0	
C1	1 (1)	0	
C2	1 (1)	0	
C3	5(7)	5(10)	
D1	2 (3)	0	
D2	4(5)	2(4)	
D3	8(11)	2(4)	
<b>STONEES</b>			
Size Inc	4(5)	4(8)	0.091
Temperature	63(84)	33(69)	0.062
Os: Probe To Bone	13 (17)	9 (19)	0.108
New Breakdown	14 (19)	2(4)	0.024
Edema, Erythema	49 (65)	22(46)	0.075
Exudate	52(69)	25(52)	0.104
Smell	34 (45)	10 (21)	0.017
<b>NERDS</b>			
Non-Healing	6(8)	3(6)	0.022
Exudate	4 (5)	6(13)	0.189
Red Friable Tissue	4 (5)	3(6)	0.107
Debris	1 (1)	4(8)	0.117
Smell	4 (5)	3(6)	0.107
<b>Presence of Deformities</b>			
No	31 (41)	31 (65)	0.010
Yes	44 (59)	17 (35)	

### 4.2.5 Diabetic Foot Ulcers of the study participants

Study participants with superficial ulcers were more likely to have a good response to HBOT (Odds Ratio = 0.3; 95% Confidence Interval: 0.1 – 0.8;  $p=0.018$ ), while those with deep ulcers were more likely to be designated as cases. The number of patients who had smelly wound when applying to the “STONEES criteria” were significantly higher in cases compared to controls. Likewise, when applying the “NERDS criteria”, cases were more likely to present with non-healing wounds than controls (see Table 4.7).

Cases were more likely to present with deformities of the foot than controls, Odds Ratio of 2.6 (95% Confidence Interval: 1.2 - 5.5). Conversely, participants who had no abnormality reported on the foot X-ray had a significantly lower risk of presenting with an inadequate response to treatment, with an Odds Ratio of 0.3 (95% Confidence Interval: 0.1 – 0.7;  $p=0.007$ ).

### 4.3 Binary logistic regression model

The binary logistic regression model is summarized in Table 4.7, while Table 4.8 indicates the model classification of cases and controls.

Step	-2 Log likelihood	Cox & Snell R-square	Nagelkerke R-square
1	107.625*	0.236	0.320

\* Estimation terminated at iteration number 5 because parameter estimates changed by less than 0.001

**Table 4.9 Regression model classification table**

Observed	Predicted		Percentage correct
	Cases	Controls	
Cases	49	11	81.7
Controls	13	27	67.5
Overall percentage correct			76.0



The variables that were included in the model are depicted in Table 4.9

							<b>95% CI for Exp (B)</b>	
<b>Variable</b>	<b>B</b>	<b>S.E.</b>	<b>Wald</b>	<b>df</b>	<b>Sig.</b>	<b>Exp (B)</b>	<b>Lower</b>	<b>Upper</b>
White Cell Count	.111	.061	3.333	1	.068	1.1118	.992	1.259
Haemoglobin	-.321	.146	4.835	1	.028	.725	.545	.966
Temperature	.073	.114	.411	1	.522	1.076	.860	1.345
STONEES (N) (missing)			4.506	2	.105			
STONEES (N) (No)	-2.633	1.269	4.304	1	.038	.072	.006	.865
STONEES (N) (Yes)	-1.692	.916	3.410	1	.065	.184	.031	1.109
STONEES (S) (missing)			1.784	1	.182			
STONEES (S) (Yes)	-.729	.546	1.784	1	.182	.482	.165	1.406
Foot deformity (Yes)	-.827	.511	2.617	1	.106	.437	.161	1.191
Constant	5.068	2.239	5.125	1	.024	158.848		

## Chapter 5: Discussion and recommendations

### 5.1 Discussion

Because DFU is commonly associated with infection, it is not surprising that a high WBC count (indicating more severe infection) is associated with poor response to HBOT.

Infectious states involving aerobic organisms are known to deplete oxygen reserves in the body and may thus actively work against the delivery of additional oxygen with HBOT in the wound area.

A number of other findings in our study was also not unexpected. It is well known that anemia increases the risk of foot complications in patients with DFU(110). Cases (individuals with a poor response to HBOT in our study) had significantly lower hemoglobin levels than controls. A low hemoglobin level in diabetic patients could be due to low iron levels, or be secondary to chronic renal failure, but may also be due to the body's response to infection. This creates difficulty in providing advice on whether this should be corrected in patients if detected at baseline, because some authors suggest that iron levels should not be corrected in patients with a current infection(111).

Inflammation is a fundamental part of the wound healing process and recruitment of neutrophils is mandatory for the clearance of microorganisms. In our study, we found that elements indicating a heightened inflammatory process were raised in the cases, including the WBC count, infrared thermometer reading, smell of the wound and new breakdown (typically due to hidden infection). This indicates that poor control of an infection causes delay in wound healing. This may be due to patient factors (such as immunity), wound care factors or an ulcer factor such as the Neutrophil Extracellular Trap (NET). NET is a natural response against infection, but excess or deregulated NETosis can cause tissue damage(112). One type of NETosis is responsible for reactive oxygen species (ROS) generation (113). One study (114) proposed that therapeutic strategies aimed to modulate NETosis should be pursued to improve the outcome in DFU patients.

Many studies have reported a positive effect of HBOT in the treatment foot ulcers in patients with DM. However, there are some studies that reported no positive effect of using HBOT in the treatment of DFU (107). This study was not performed to determine whether HBOT is effective as an adjunctive treatment for Diabetic Foot Ulcer (DFU), but was performed in the

context of patients who had already been selected for HBOT using standard international selection criteria, mainly based on Transcutaneous Oxygen Monitoring (TCOM) results.

Within this context, there are very few studies that assessed the factors that may influence wound healing and the response to HBOT in patients with DFU (108) (109). For this reason, the objective of the current study was to determine potential factors that may influence wound healing response when using HBOT in patients with DFU.

When considering the conditions that define an “inadequate response to HBOT” in the study, it was surprising to see the high proportion of cases compared to controls, amounting to 60.98% of the study participants. The majority of these cases were due to less than 30% closure of the wound area, while some developed new ulcers and/ or required an amputation at the level of the ankle or above. This high percentage of “inadequate responses” highlights the need for improved selection of patients for HBOT. It would also explain why some authors question the value of HBOT for DFU(107). These findings should however be interpreted with caution, as highlighted in the section below.

A number of anthropometric and laboratory measurements are routinely performed on patients in the unit. It was surprising to see that very few of these could be used to predict the response to HBOT. None of the demographic or anthropometric measurements could predict the outcome of the treatment. An increased body mass index would suggest a higher pressure on an ulcer when the patient is walking, but this seemed not to be an important factor in our study.

We found that a high white blood cell (WBC) count, a low hemoglobin (Hb) level, and high infrared thermometer readings were baseline tests that were statistically associated with the cases. Upon clinical examination, we likewise found that new wound breakdown, chronic, non-healing wounds and increased smell of a wound to be factors associated with an inadequate response to HBOT. The combination of these factors seem to point to ongoing infection and inadequate response to antimicrobial therapy to be the likely cause for poor treatment response.

Neither age nor gender was related to the outcomes in our study. These findings are consistent with the findings of a previous study(115). However, there are studies that have found an association with age and demonstrated that patients who achieved better outcomes were younger (116).

A history of uncontrolled diabetes results in a high number of patients with diabetic complications such as peripheral arterial disease, neuropathy or nephropathy. As expected in a diabetic population, a large proportion of the patients in our study received angioplasty before presenting at our unit or required angioplasty as part of their treatment. Peripheral arterial disease is an important risk factor for impaired wound healing and lower extremity amputation(117). Hokkam assessed the impact of risk factors on the outcome of diabetic foot ulcers and identified that peripheral arterial disease is significantly related to the development of ulceration but not to the ultimate outcome of the wound (118)

The Wagner grading of ulcers is commonly used in the assessment of patients with DFU for HBOT. Previous studies observed a poorer outcome in patients with Wagner grade 3 classification or above. Our study findings were consistent, but not statistically significant (119). The reason for the findings in our study is most likely based on a selection bias, since Wagner grade 3 and above would typically be the type of patients that will be selected for HBOT at the unit.

The number of treatments received was not found to be significantly related to the ultimate outcome in our study. This is in contrast to a study that observed that 73.8% of patients improved with a mean number of 34 HBO treatments and that patients who did not improve received a mean number of 24 HBO (119). Another study proposed that 30 – 40 HBOT sessions are common for DFU. The Undersea and Hyperbaric Medical Society do not recommend a specific number of treatment sessions, but they do advise practitioners to re-evaluate the patient and the wound after 30 days of treatment and to proceed with further treatment sessions if indicated. Our study likely did not find a significant difference in the number of treatment sessions because standardized unit protocols are used in the treatment. It is however important to interpret the association between the number of treatment sessions and healing as an outcome with caution. A low number of treatment sessions may be associated with a poor outcome because of a poor response overall and a clinical decision to stop treatment. On the contrary, a high number of treatment sessions may be associated with a poor response when clinicians opt to continue with HBOT despite evidence that there is no response. It is therefore important for future studies to identify objective measures that would guide clinicians in advising additional treatment sessions beyond the proposed 30-40 HBOT sessions.

In conclusion, this study points out factors that influence wound healing in patients receiving HBOT as part of their plan of management. These findings show anemia and infection level

are very crucial to improve the outcomes in DFU patients. Of these, it seems like aggressive management of infections is important to ensure an adequate response to HBOT.

## 5.2 Strengths and limitations of the study

One of the strengths of our study is that the hyperbaric facility has a world-class advanced wound care unit. Both of these units have standardized approaches to patient selection and patient care and make use of standardized documents to capture patient information and guide their management. This allowed for the availability of rich patient data. Nonetheless, the information that were captured had the clinical management of the patients in mind, rather than future research and (like all retrospective studies) our study could be subject to information bias. Information that the clinical personnel may not have thought to be important in the clinical management of the patient may thus be omitted from the clinical notes, including potential important negative findings in the patient histories. Patients may also present their history in general terms and approximations. For instance, patients may round the duration of their illness up or down and not provide the exact figure. For this reason, our study included objective measures obtained during the initial consultation, including laboratory results and the objective wound assessment protocols.

Some data was missing on individuals that may have been included in the study. It is unclear whether this information is more likely to be missing in cases or controls. Informal discussion with the unit personnel seem to indicate that this may be the case. Positive findings are generally listed for individuals who do not respond well, while patients who are discharged from the unit (following successful treatment), may not have this information specifically mentioned in their files. The fact that outcome data was missing for a large number ( $n=40$ ) individuals may explain the high number of cases (compared to controls) in our study.

The selection of controls is an important consideration in all case-control studies. Our study used all available patients as study participants. This would normally reduce the risk of selection bias. However, our selection was based on the availability of specific outcome information and if the missing data is associated with the outcome, there may be a systemic error in our study findings.

Despite the weaknesses mentioned above, this study also had a number of strengths. The clinicians involved in the treatment of the patients were blind in terms of the final outcome of

the patients at the time of measuring the independent variables. The unit also utilizes standardized documentation when examining and treating patients. This is the case for the HBOT facility, as well as the wound care unit. Many of the measurements are based on objective findings, including laboratory tests. The risk of measurement bias is thus very low in this study.

The classification of cases and controls were based on objective measurements, including the measurement of wound sizes using a 3-dimensional camera with software that plots the progress with each visit. New wounds and amputations are also objective findings used for classification. The risk for misclassification bias is therefore considered to be low.

### **5.3 Recommendations**

One of the major factors that would contribute to increase the risk of infection in diabetic patients is poor glycemic control. A strict control of blood sugar is recommended for the treatment of DFU. Also, patients with infrared reading of three degrees or more deserve to be admitted for an intravenous antibiotic before the infection presents clinically in form of redness or induration.

Anemia which could be difficult to treat, should be addressed before commencing the treatment. Simple iron deficiency anemia can be treated with iron replacement therapy. However, patients with anemia secondary to chronic kidney disease should be referred for erythropoietin treatment.

The usefulness of HBOT in the treatment of DFU has not yet been fully elucidated and it is possible that additional factors may be used in the assessment of patients in order to improve patient selection. Our study suggests that patients who are selected for HBOT in accordance with existing patient selection criteria as recommended by the UHMS can be stratified further by focusing particularly on infection in the wound and to ensure that this is adequately addressed. Ongoing infection seems to be a likely explanation for a poor response to HBOT.

### **5.4 Further studies**

Our study did not measure long-term outcomes of patients receiving HBOT for DFU. Future prospective studies should consider long-term follow-up of patients to determine whether HBOT has any long-term benefits for patients and how long the benefit would last.

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