Risk Factors of Lower Extremity Amputation in Diabetic Foot Ulcer Patients: A Hospital Based Case-Control Study

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Taguhi Sevoyan, MD, MPH candidate

Advising Team: Vahe Khachadourian, MD, MPH, PhD

Lisle Hites, MS, MEd, PhD

Turpanjian School of Public Health

American University of Armenia

Yerevan, Armenia

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List of Abbreviations

DFU	Diabetic foot ulcer
DM	Diabetes mellitus
LEA	Lower extremity amputation
MC	Medical Center
PAD	Peripheral artery disease
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus

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Abstract

Background: Diabetic foot ulcers have reached epidemic proportions, and constitute significant public health and economic burden for health systems worldwide. The global prevalence of diabetic foot ulcers demonstrates considerable variation with a pooled prevalence of 6.3%. Lower extremity amputations are among the most severe and life-threatening health complications of diabetic foot, leading to reduced quality of life and increased medical costs. Diabetes-related foot ulcerations remain the principal cause of non-traumatic lower extremity amputations worldwide.

Objectives: The study sought to identify the risk factors associated with amputation in diabetic foot ulcer patients as well as to identify the risk factors associated with major amputation in patients with diabetes-related amputations in Armenia.

Methods: A case-control study design was utilized involving patients admitted to Armenia Medical Center and Erebouni Medical Center during the year of 2018. Cases were defined as patients who were admitted to the medical center and underwent minor or major lower extremity amputation. Controls were patients who had diabetes-related hospitalization and treatment with coexisting diabetic foot ulcer. Data on 52 potential risk factors were collected from the patients via telephone interviews and medical records reviews. To assess the strength of the association between the potential risk factors and lower extremity amputation simple and stepwise multiple logistic regression analyses were conducted.

Results: The study comprised 77 cases and 77 controls. Simple logistic regression analysis revealed statistically significant differences between LEA and DFU groups in 7 out of 52 potential risk factors. In a stepwise multiple logistic analysis, three (age 51-60 years (OR=17.86; 95% CI: 1.57-202.28) vs. <50 years, age >70 years (OR=68.58: 95% CI: 5.08-924.66) vs. <50 years, history of diabetic foot ulcer (OR=123.24; 95% CI: 13.15-1154.65) and total leukocyte count (OR=1.37; 95% CI: 1.15-1.64)) of the 7 risk factors remained significant. Similarly, statistically significant differences between major LEA and minor LEA groups were observed in diabetes treatment involving insulin therapy combined with oral agents and diet (OR=0.02; 95% CI: 0.0009-0.48), total leukocyte count (OR=1.19; 95% CI: 1.05-1.35) and fasting plasma glucose levels (OR=0.85; 95% CI: 0.76-0.96).

Conclusion: The risk factors of lower extremity amputation among hospitalized diabetic foot ulcer patients were age, history of foot ulcer and total leukocyte count. Additionally, the risk factors of major LEA among patients with diabetes-related LEA were diabetes treatment, total leukocyte count and fasting plasma glucose.

1. Introduction

1.1 Background

Diabetic foot is described as a foot affected by ulceration, infection and/or deep tissue destruction that is associated with various degrees of peripheral neuropathy and/or peripheral artery disease of the lower extremity in patients with diabetes mellitus (DM).[1] DM is a complex disease in which the pancreas is not synthesizing insulin properly or when the organism's response to the hormone is impaired.[2] Diabetes is classified into two main types. Type 1 diabetes mellitus (T1DM), widely known as juvenile diabetes, as it is generally detected in children and adolescents, described as a condition when very low or no insulin is produced resulting in insulin dependency.[3] Type 2 diabetes mellitus (T2DM) is the most prevalent form of the disease, widely known as non-insulin dependent diabetes, is specified as a condition of relative insulin deficiency as well as resistance to insulin that leads to hyperglycemia.[4] Consistently hyperglycemia can lead to microvascular and macrovascular complications causing a wide range of serious health problems.[5] Classic microvascular complications involve neuropathy, nephropathy, retinopathy; however encephalopathy, foot complications, skin infections and periodontitis are also possible.[6,7] Macrovascular pathologies include atherosclerotic and thrombotic occlusions in coronary, cerebral and peripheral arteries increasing the risk of peripheral artery disease (PAD), cardiovascular disease and stroke in diabetic patients.[7,8]

Diabetic foot ulcers (DFUs) represent a serious chronic complication of DM progression.[9] DFU is a multifactorial clinical issue, comprising physical, behavioral and biomechanical factors.[10,11] Major contributing causative factors to foot ulceration are peripheral neuropathy and PAD.[12] Peripheral neuropathy occur as a result of metabolic abnormalities due to hyperglycemia and lead to impaired motor innervations (motor neuropathy), loss of sensation in

lower extremities (sensory neuropathy) and nerve damages in parasympathetic and sympathetic nervous system (autonomic neuropathy).[13] PAD is the obstructive atherosclerosis of arteries, characterized by severe ischemic conditions of the lower extremity.[14,15]As a result, patients with diabetes may develop a chronic non-healing foot ulcer and undergo a lower extremity amputation (LEA) which is associated with increased morbidity and mortality.[16]

1.2 Global prevalence/incidence

DFUs have reached epidemic proportions in the global population and pose significant public health burden. According to a systematic review and meta-analysis of studies published until September 2015 done by Zhang et al, the pooled prevalence of DFUs was around 6.3% worldwide and was higher in men (4.5%) than in women (3.5%) and higher in T2DM patients (6.4%), if compared with T1DM (5.5%).[17] The highest prevalence is reported in the North America (13%) followed by Africa, Asia, Europe and Oceania, 7.2%, 5.5%, 5.1% and 3.0% respectively.[17] The prevalence of DFUs varies greatly on a global scale ranging from a low of 1.5% in Australia to a high of 16.6% in Belgium, followed by 14.8% in Canada and 13.0% in the US.[17] In Europe, Norway had a prevalence of 10.4%, Italy 9.7% and Denmark 7.8%.[17] Significant heterogeneity was observed in prevalence rates among Asian countries ranging from 11.6% in India, 8.8% in Thailand to 2.0% in Japan and 1.7% in Korea.[17] Another systematic review and meta-analysis on the prevalence of foot and ankle disorders in general inpatient populations reported the pooled prevalence estimates of DM related foot disorders 4.7%, DM related foot infections 3.4% and DM related foot wounds 2.4%.[18] In Canada, a retrospective analysis of diabetes-related health issue documented in four national databases revealed 25,597 prevalent and 14,449 incident cases of foot ulcerations.[19] The prevalence rate ranged across the country with the highest prevalence of 8,552 cases in Ontario followed by 6,265 and

3,560 cases in Quebec and British Columbia respectively.[19]According to a systematic review of studies conducted in five Arab countries, the mean prevalence of DFU was 11.85% in Saudi Arabia, 5.9% in Bahrain, 4.65% in Jordan, 4.2% in Egypt and 2.7% in Iraq.[20] A meta-analysis on the prevalence and health outcomes of DFUs in Africa revealed a mean prevalence of 13% with high heterogeneity across DFU prevalence estimates, the highest of 16.7% was reported in Central Africa followed by 16.4% in Western Africa, 12.6% in Northern Africa, 11.9% in Eastern Africa and the lowest of 4.6% in Southern Africa.[21] The same study indicated that Africa had highly heterogeneous major amputation prevalence rates ranging from 3.8% in Southern Africa to 20.0% in Central Africa with a pooled prevalence of 15.5%.[21]

Lower extremity amputations are among the most severe and life-threatening health complications of diabetic foot, leading to reduced quality of life and increased medical costs. Of all non-traumatic LEAs, up to 75% are attributable to DM.[22,23] The first most common indicator preceding non-traumatic LEAs related to DM is foot ulceration in 85% of cases.[24] According to a review analysis by Narres et al, the global incidence of lower extremity amputations varied between 78 and 704 per 100,000 person-years in people with diabetes and the relative risks ranged between 7.4 and 41.3 in diabetic and non-diabetic patients respectively.[23] A meta-analysis of studies on the incidence of LEA published from 1989 until 2010, demonstrates a significant variation in all forms of LEAs between diabetic and non-diabetic populations, 4.6-9600 and 5.8-31 per 100,000 persons, respectively.[25] A review of population-based studies published between 1988 and 2011, reported a tendency of decreasing diabetes-related complications worldwide.[26] A nationwide study conducted in Germany (2005-2007) revealed gender differences in incidence rates per 100,000 person years between diabetic and non-diabetic patients; 176.5 and 20.0 in males vs. 76.9 and 13.4 in females, respectively.[27] Another

nationwide study, covering approximately 34 million inhabitants, reported a significant reduction in amputation rates per 100,000 person-years from 81.2 to 58.4 for major LEAs and from 206.1 to 177.0 for minor LEAs.[28] In contrast, a retrospective hospital-based study conducted in Singapore, revealed an increase in major and minor amputation rates per 100,000 people from 2008 to 2013, from 11.0 to 13.3 and 10.8 to 13.9 respectively.[29] A nationwide study in England suggests that the incidence rates per 10,000 persons of all forms of amputations decreased from 27.5 in 2004 to 25.0 in 2008 in people with diabetes.[30]

1.3 Financial burden

DFU represent a significant economic burden for health systems globally. In the US DFUs treatment adds an additional US $9-13 billion to the annual diabetes-related medical expenses for public and private payers.[31] Medical expenditures for DFUs treatment were assessed to be US $11,710 and US $16,883 per patient for Medicare and private insurance companies respectively.[31] In the UK DFUs treatment costs £580.5m to the National Health Service which was approximately 0.6% of total health expenses.[32] In Canada, the total cost of DFU-related care represented $547.0 million or $21,371 per patient annually, where $320.5 million accounted for acute care, in addition to $125.4 million and $63.1 million for home care and long-term care respectively.[19] Despite all the possible actions taken to combat diabetes-related costs, the global financial burden of the disease is expected to continuously increase through 2030.[33]

1.4 Risk factors

Various studies investigated lower extremity amputations in sequelae of diabetes. Numerous studies reported peripheral neuropathy, peripheral artery disease and infection as underlying causative risk factors for ulcer formation and diabetes-related amputations.[34–40]

A prospective hospital-based study following 1,461male diabetic patients for 22 years from Seattle, WA identified sensory neuropathy (HR=3.09), poor vision (HR=1.7), body weight (HR=0.78) and age over 70 years vs. age less than 57 years (HR=0.13) as factors associated with a significant risk of LEA.[38] Another prospective study conducted in 10 European countries among 575 infected DFU patients revealed ulcer depth, osteomyelitis and purulent exudate as significant factors that may independently predict LEA.[37] A seven-year prospective study of diabetes-related LEAs conducted in Costa Rica reported an increased risk of amputation is also associated with male gender (HR=3.81), diabetes duration (HR=1.08), insulin therapy (HR=10.95) and history of LEA (HR=16.58).[40] A hospital-based longitudinal study investigating the risk factors of LEA in infected DFU patients suggests a clear link between fasting plasma glucose level, osteomyelitis, peripheral neuropathy, angiopathy and significantly higher risk of amputation.[39] Increased risk of amputation is also associated with hypertension (OR=0.09) according to a retrospective analysis of factors determining the risk of LEA.[41] Another nationwide retrospective analysis of Veteran Healthcare Administration files from 1998 to 2000 reported female gender and marital status as significant factors associated with amputation in diabetic patients.[42] Korea University Guro Hospital case-control study reported that dialysis (OR=8.68), ulcers penetrating into the bone (OR=11.67), hind foot ulcers (OR=6.15), hemoglobin levels (OR=0.64) and fasting plasma glucose levels (OR=1.007) are significantly associated with increased risk of amputation in diabetic patients.[43] Several hospital based case-control studies reported nephropathy, retinopathy, stroke and low hematocrit levels as significant factors that may independently predict LEA.[44–47]

1.5 Classification systems

DFU classification systems represent a crucially important decision-making and prognostic tool for LEA occurrence. Various systems attempted to classify DFUs for LEA prediction; however, no classification system has up to the present time been accepted as gold standard.[48]

The Wagner classification system, developed in 1981 by William Wagner, is one of the most commonly used and highly validated tools to apply for DFUs classification.[49,50] The Wagner classification system is based on penetration depth in the affected region, the presence or absence of osteomyelitis or gangrene, and the gangrene progression.[49,51] The Wagner classification includes 6 grades, that are described as follows:

"0 = no open lesions; may have deformity or cellulitis

1 = superficial diabetic ulcer (partial or full thickness)

2 = ulcer extension to ligament, tendon, joint capsule, or deep fascia without abscess or osteomyelitis

3 = deep ulcer with abscess, osteomyelitis, or joint sepsis

4 = gangrene localized to portion of forefoot or heel

5 = extensive gangrenous involvement of the entire foot." [51]

1.6 Situation in Armenia

DM is approaching epidemic proportions in the Republic of Armenia. According to International Diabetes Federation data, the prevalence of diabetes in adults was 7.6% affecting 168,400 people in 2017.[52] Whereas World Health Organization reported a total prevalence of diabetes of 12.3%, all ages (11.1% in males vs. 13.5% in females) in 2016.[53] Diabetes remains the second cause of years lived with disability with an increasing tendency (2.2%) from 2007 to

2017.[54] In 2018, non-communicable diseases accounted for 93% of all deaths, out of which 4% was attributable to diabetes.[55] Diabetes accounted for 520 (300 in males vs. 220 in females) and 730 (270 in males vs. 460 in females) deaths in 30-69 and 70+ age groups respectively in 2016.[53]

Additionally, the lack of studies investigating the risk factors of amputations among individuals with DM in Armenia leads us to conduct interviews with healthcare professionals in hospitals for a preliminary assessment of the disease burden. According to physicians, the number of patients in advanced stages of the disease, as well as, the number of amputations due to poor patient adherence continuously increases annually, imposing significant public health burden in Armenia. The current study was conducted to eliminate the gap in the literature.

1.7 Investigation rationale

A systematic review and meta-analysis on epidemiology of DFUs indicate that this is a widespread condition and a severe public health issue in many countries, however, there is a scarcity of studies investigating the global epidemiology of this complication of diabetes.[17] Though substantial research was conducted on the pathogenesis of DFUs and the causal pathways to foot amputations due to diabetes, there has been a lack of studies investigating the risk factors responsible for different types of lower extremity amputations. However, further investigation may provide new evidence that could help to design better and more effective ways to manage the disease and improve the quality of life. Although many treatment options are available, patient lifestyle is vital in improving diabetes management. The current study may generate knowledge about the behavioral factors that contribute to lower limb amputation by exploring the connection between lifestyle habits and the disease outcomes. Additionally, the research may develop an understanding of various factors leading to amputations and whether the findings can be exploited in clinical medicine.

1.8 Research questions

- What risk factors are associated with lower extremity amputation in patients with diabetic foot ulcers in Armenia?
- What risk factors are associated with major lower extremity amputation in patients with diabetes-related lower extremity amputations in Armenia?

2. Methods

2.1 Study design

A case-control study design was utilized as it is efficient for investigating multiple exposures and rare outcomes. A total of 2 groups were selected.

The research participants were selected from the Armenia Medical Center (MC) and Erebouni Medical Center (MC).The selected patients were contacted for obtaining an informed consent and scheduling telephone interviews prior to extracting information from the medical records.

2.2 Study population

Target population: Patients aged ≥18 years with either T1DM or T2DM and coexisting DFU, residents of Armenia.

Source population: Patients aged ≥18 years with either T1DM or T2DMhospitalized for DFU treatment at either Armenia MC or Erebouni MC during the year of 2018.

Cases are defined as patients aged ≥18 years with either T1DM or T2DM and coexisting DFU, who underwent minor (below the ankle) or major (above the ankle) LEA during the year of 2018.

Controls are defined as patients aged ≥18 years with either T1DM or T2DM and coexisting DFU (at least stage 1 based on Wagner classification), who were hospitalized for surgical debridement or non-surgical treatment during the year of 2018.

Exclusion Criteria

Patients with chronic venous insufficiency, varicose veins, acute peripheral artery thrombosis, congestive heart failure, lymphedema, rheumatological diseases (vasculitis, scleroderma, systemic lupus erythematosus, etc.), or malignant neoplasm, or patients who receive radiotherapy and immunosuppressive therapy were excluded from the study because of the possibility of lower extremity ulceration and amputation due to other health conditions than diabetes.[56] Additionally, patients with ulcers and amputations due to other reasons than DM and patients who are not residents of Armenia and/or can't speak Armenian were excluded from the study.

2.3 Sample size

The sample size calculation was based on the considerations about the prevalence of different possible risk factors in patients who are free of amputations and odds ratios for LEAs associated with those risk factors (Appendix 1).

The sample size calculation was performed manually using a formula for the difference in proportions (Appendix 2). These calculations were confirmed using STATA software. It was proposed to get a sample size that will allow to detect odds ratio of 2.5 if the prevalence of the risk factor is 40%, with a level of significance 0.05, study power of 0.8 and the ratio of controls to cases equal to 1.0. The selected values are conservative with enough power to detect associations for most predictors of LEA, taking into account feasibility issues. The total sample size was computed to be 154, with 77 participants in each group.

2.4 Data collection and study instrument

After getting the permissions for conducting the research from the medical centers, data collection was performed. All patients, identified from the corresponding departments' journals, who meet the inclusion criteria, formed the sampling frame. Medical records were sorted by the presence of performed LEA or hospitalization for reasons related to DM during the year of 2018. For the selection of cases, systematic random sampling was applied to obtain a sample of 77 patients. Simple random sampling was used to select the study participants for controls from patients who had diabetes-related hospitalization. Controls were frequency matched to cases on the medical center and the year of hospitalization. Corresponding medical data and patients' contact information was derived from the medical records.

Afterwards, the patients were contacted to get the informed consent, were passed through the screening procedure and were administered to the corresponding group accordingly and to arrange the interviews.

Interviewer-administered questionnaire was applied for the data collection using telephone interviewing mode. The student investigator framed the questionnaire by adapting questions used in other studies on DFUs, questions that were extracted from instrument utilized in household health survey conducted in Armenia, and questions developed by the researcher (Appendix 3 and 4). The questionnaire involves screening questions for identifying eligible participants and the following domains: family history, lifestyle, additional questions, socio-demographic and anthropometric characteristics, and the medical record review form. Smoking habits, alcohol consumption and socio-demographic characteristics were evaluated using questions adopted from the instrument used in household health survey conducted in Armenia.[57] The question on patient's preferred footwear was adopted from the North West Diabetes Foot

Study.[58] Questions related to patient's family history, questions on glucometer use, flatfoot and the medical record review form were developed by the investigator.

2.5 Study variables

The dependent variable is whether the patient had a confirmed LEA (in the medical record and verified by the patient) during the year of 2018.

Independent variables are family history, smoking status, alcohol consumption, glucometer use, flatfoot, preferred footwear, socio-demographic and anthropometric characteristics (residency, gender, age, body mass index, education, employment, marital status, household monthly expenses). Additional information was derived from medical records (diabetes type, diabetes duration, diabetes treatment, history of foot ulcer, history of surgical treatment, comorbidities, ulcer depth, ulcer location, ulcer level, wound infection, foot abscess/flegmona, osteomyelitis, hemoglobin, total leukocyte count, erythrocyte sedimentation rate, fibrinogen, total protein, fasting plasma glucose, blood urea nitrogen, serum creatinine, urine PH, urine protein, urine glucose, ketone bodies in urine) to assess the association between these predictors and the outcome.

2.6 Data management and analyses

Double data-entry followed by data cleaning through sorting and spot-checking was performed with IBM SPSS Software. The collected data was imported from SPSS to STATA for data analysis.

Descriptive statistics was used to summarize characteristics of the cases and controls. Means and standard deviations were calculated to describe continuous variables, and frequencies were used to describe categorical variables. The distribution of continuous and categorical variables in cases and controls were compared using t-test and Chi-square test respectively. The

strength of the associations between potential predictors and the outcome were determined through simple and stepwise multiple logistic regression analyses. Variables with more than 10% of missing values were excluded from regression models. P-value of less than 0.05 was considered as statistically significant.

2.7 Ethical considerations

The study was conducted considering all the privacy and confidentiality issues. The Institutional Review Board (IRB) at the American University of Armenian reviewed and approved the study protocol. Requests of permission for conducting the research were sent to the medical centers' General Directors. After obtaining the IRB approval and permissions from the medical centers, access to medical records was granted. Patients were contacted and if eligible were asked for oral informed consent before starting the interview (Appendix 7 and 8). All participants voluntarily agreed to participate and gave an oral consent prior to the interview. Participants were informed about their participation in the study on risk factor of amputation due to DFUs. No incentives or compensations were used to encourage the participation in the project. Participants were informed that they can skip any question and refuse to continue the interview at any time. Participants' personal information was available only to the student-investigator and didn't serve other motives. All participants were provided with a telephone number for contacting the research team in case of any issues or questions about the study.

3. Results

3.1 Response rate

The study intended to involve 77 patients in each group. Purposely, a total of 248 and 550 potentially eligible patients were identified for cases and controls respectively from either

medical center. After eliminating ineligible patients, list of potential eligible cases and controls were 133 and 148 respectively. Afterwards, the remaining patients were approached to complete the sample size with 77 participants in each group. Ultimately, 79 out of 133 eligible patients and 86 out of 148 potential controls had required valid contact information available and so were contacted. The response rate was 97.46% for cases and 89.53% for controls, calculated out of contacted patients who meet the eligibility criteria. The study sample selection details are presented in the Figure.

3.2 Descriptive statistics

From a total of 77 diabetic patients who had lower extremity amputation, 29.87% (n=23) were from Erebouni MC and 70.13% (n=54) were from Armenia MC. Equivalent number of controls to cases was involved from each medical center. Major and minor amputations composed 48.05% (n=37) and 51.94% (n=40), respectively of LEA cases. Most of the major (28 out of 37) and minor (26 out of 40) amputations were performed in Armenia MC.

LEA patients were predominantly male, comprising 63.63% of the sample (23 patients in major LEA and 26 patients in minor LEA patients) in comparison to DFU patients where the population was majorly consisted of female. The estimated average age at hospitalization was higher in cases compared to the controls with mean age of 66.6±8.3 (68.7±7.7 for the major LEA and 64.7±8.4 for minor LEA patients) versus 56.2±10.0 respectively.

The majority of LEA patients reported ever smoking (54.55% vs. 40.26% of the controls). At the time of the interview, about 30% of ever smokers in the cases and 58% of ever smokers in the controls reported smoking every day, while 50% vs. 61% of ever smokers in the cases and controls reported smoking before the hospitalization respectively. The average number of cigarettes smoked a day was 19.1±10.8 in cases vs. 21.7±14.1 in controls, compared to

28.0±18.3 in cases vs. 24.7±22.7 in controls before the hospitalization. LEA patients reported 30.2±14.4 years of smoking during their lifetime compared to 21.4±13.6 years in DFU patients. Lifetime alcohol consumption differences were also observed across the groups: about 56% of cases reported a certain extent of alcohol use compared to 43% in controls. More than 21% of major LEA patients reported not having a glucometer before the surgery, thus not measuring blood glucose level on their own, in comparison to 12.5% in minor LEA group. Detailed socio-demographic and lifestyle characteristics by cases and controls are provided in Table 1.1.

Study participants primarily had type 2 diabetes (147 out of 154) where 7 individuals with type 1 diabetes were in the control group. Diabetes duration was on average higher in amputation group with a mean of 16.4 years and a standard deviation of 8.6 versus 11.7 years and a standard deviation of 6.7. The majority of cases had insulin with or without oral agents in their diabetes treatment plan (71.42% vs. 64.93% for the controls). About 60% of the cases had only insulin therapy compared to 45% in controls. History of diabetic foot ulcer was almost 23 times more prevalent in cases (59.74% vs. 2.60% in controls). About 38% of LEA patients reported a history of either surgical debridement, minor or major amputation, whereas none of the controls ever had a surgery for diabetic foot ulcers. Ischemic heart disease prevalence was the highest among major LEA patients (59.46%) followed by 49.35% in minor LEA and 35.00% in DFU patients. About 16% of cases had a history of myocardial infarction compared to 9.09% in controls. Cases also were found to have a history of stroke more often (5.19%) than controls (1.30%).

Differences in ulcer characteristics were also observed across the groups. Most of the ulcers were located on the border of feet (90.00% in minor LEA group and 92.21% in DFU group). Forefoot ulceration was the most common ulcer level with around 95% prevalence in

minor LEA and DFU patients. Ulcer penetration to bone, infection, abscess/flegmona and osteomyelitis were present in all LEA patients making them perfect predictors of amputation.

Blood serology test results showed significantly high levels of inflammation in LEA patients, in contrast DFU patents revealed low-grade or no inflammation. Total protein and serum Creatinine levels were higher in major LEA patients (94.6±135.4 vs. 74.0±6.5 in minor LEA vs. 75.5±7.2 in DFU patients and 109.5±57.6 vs. 91.3±34.8 in minor LEA vs. 95.7±76.2 in DFU patients respectively), as well as blood urea nitrogen levels (9.2±4.3 vs. 7.9±4.6 in minor LEA patients vs. 7.3±4.2 in DFU patients), indicating differences in kidney function between the groups. Urine tests also revealed higher urine protein (85.11% vs. 53.25% in controls) and ketone bodies (33.33% vs. 15.79% in controls) level in amputation group. Table 1.2 presents descriptive statistics on clinical and laboratory characteristics by case-control status.

3.3 Simple logistic regression analysis

The results of simple logistic regression analysis to assess the strength of the association between LEA and independent predictors are reported in Tables 2.1 and 2.2. The analysis revealed that males had significantly higher odds of amputation in comparison with females, OR=1.99 (95% CI: 1.04-3.79; p=0.0347). Participant's age was significantly associated with the risk of amputation; patients aged 61 to 70 and >70 years had 13.30 (95% CI: 2.80-63.04; p=0.001) and 39.51 (95% CI: 6.75-226.06; p<0.0001) times higher odds of amputation in comparison with patients aged 50 years and younger respectively. Smoking duration was significantly associated with the risk of LEA showing that the odds of amputation increased by 3% (OR=1.03; 95% CI: 1.00-1.05; p=0.0028) with each year increase in smoking duration. History of diabetic foot ulcer was significantly associated with the risk of amputation; those with previous foot ulcer had 55.64 times higher odds (95% CI: 12.71-243.53) of amputation compared

to those without previous foot ulcer. Hemoglobin level was negatively associated with the risk of amputation indicating that each g/L increase in hemoglobin level decreases the odds of amputation by 4% (OR=0.96; 95% CI: 0.95-0.98 p=0.0004). Total leukocyte count (OR=1.38; 95% CI: 1.21-1.58; p<0.0001) and erythrocyte sedimentation rate (OR=1.05; 95% CI: 1.03-1.07; p=<0.0001) were statistically significantly associated with the risk of LEA. Ulcer depth, infection, abscess/flegmona and osteomyelitis were not included in the simple and multiple logistic regression models, since those factors are clinically strongly correlated with lower extremity amputation. Household monthly expenses were also excluded from the model as no information was available on the household size.

In summary, statistically significantly results were obtained by gender, age, smoking duration, history of diabetic foot ulcer, hemoglobin count, total leukocyte count and erythrocyte sedimentation rate in LEA and DFU patients.

Tables 3.1 and 3.2 show the results of simple logistic regression analysis for potential predictors of major lower extremity amputation. The analysis revealed that, the estimated OR between participant's age and risk of LEA was 3.63 (95% CI: 1.06-12.40; p=0.039) showing that patients aged 61 to 70 years had 3.63 times higher odds of amputation compared to patients aged 50 years and younger. Body mass index was another significant risk factor for amputation (OR=0.89; 95% CI: 0.080-0.99; p=0.0334).Family history of LEA was statistically significantly associated with the risk of amputation (OR=0.22; 95% CI: 0.004-1.15; p=0.0491). Compared to patients who followed diabetic diet plan, patients who received oral agents, insulin therapy and combined therapy with insulin and oral significantly had lower odds of amputation, (OR=0.07; 95% CI: 0.007-0.82; p=0.034, OR=0.10; 95% CI: 0.01-0.90; p= 0.041 and OR=0.06; 95% CI: 0.005-0.76;p=0.030)respectively. The estimated OR between ischemic heart disease was 2.72

(95% CI: 1.08-6.85; p=0.0308) showing that patients with ischemic heart disease had 2.72 times increased odds of amputation compared to those without ischemic heart disease. Total leukocyte count was significantly associated with the risk of LEA indicating that each10^9/L increase in total leukocyte count increases the odds of amputation by 12% (OR=1.12; 95% CI: 1.02-1.22; p=0.0089). The estimated OR of fasting plasma glucose associated with amputation was 0.90 showing that each mmol/L increase in fasting plasma glucose decreases the odds of LEA by 10% (OR=0.90; 95% CI: 0.82-0.99; p=0.0316).

Ultimately, major LEA and minor LEA patients were statistically significantly different by age, body mass index, family history of diabetes-related lower extremity amputation, diabetes treatment, ischemic heart disease, total leukocyte count and fasting plasma glucose.

3.4 Multiple logistic regression analysis

Multivariable logistic regression analysis was run to estimate the odds of LEA for gender, age, smoking duration, and history of diabetic foot ulcer as categorical predictors and hemoglobin count, total leukocyte count, erythrocyte sedimentation rate as continuous predictors, to produce the final model. After removing all non-significant variables, the final model encompassed age, history of diabetic foot ulcer, total leukocyte count and erythrocyte sedimentation rate (Table 4). The participant's age was significantly associated with higher odds of LEA; patients aged 61 to 70 and >70 years had 17.86 (95% CI: 1.57-202.28; p=0.020) and 68.58 (95% CI: 5.08-924.66; p=0.001) times higher odds of amputation compared to patients aged 50 years and younger. Patients with a history of foot ulcer had significantly higher odds of amputation (OR=123.24; 95% CI: 13.15-1154.65; p<0.0001) compared to patients without foot ulcer. The estimated OR of total leukocyte count associated with LEA was 1.37 showing that

each 10^9/L increase in total leukocyte count increases the odds of amputation by 37% (OR=1.37; 95% CI: 1.15-1.64; p<0.0001).

Body mass index, total leukocyte count, fasting plasma glucose as continuous variables and age, family history of diabetes-related lower extremity amputation, diabetes treatment, ischemic heart disease as categorical variables were included in the multivariable model. The final model comprised diabetes treatment, total leukocyte count and fasting plasma glucose after removal of all non-significant variables (Table 5).The analysis showed that the type of received treatment for diabetes was significantly associated with the risk of LEA; patients who receive combined therapy with insulin and oral agents along with diet had 0.02 times lower odds (OR=0.02; 95% CI: 0.0009-0.48; p=0.016) of amputation compared to those patients who follows diabetic diet plan only. Total leukocyte count was associated with higher risk of LEA showing that each 10^9/L increase in total leukocyte count increases the odds of amputation by 19 % (OR=1.19; 95% CI: 1.05-1.35; p=0.006). Fasting plasma glucose level was negatively associated with the risk of amputation indicating that 1 mmol/L increase in fasting plasma glucose level decreases the odds of LEA by 15% (OR=0.85; 95% CI: 0.76-0.96; p=0.010).

4. Discussion

4.1 Main findings

The presented case-control study investigated the risk factors associated with lower extremity amputation as well as risk factors associated with major lower extremity amputation in patients with diabetes mellitus in Armenia. The study revealed a statistically significant association between diabetes-related LEA and age, history of foot ulcer, total leukocyte count and erythrocyte sedimentation rate. Additionally, statistically significant differences were found

between major and minor LEA groups in diabetes treatment, total leukocyte count and fasting plasma glucose levels.

In general, the study findings were consistent with the international literature. Age and gender were identified as a predictive factor of amputation in the simple analysis. In the multiple model, age maintained statistical significance. A prospective study conducted by Chen et al., that enrolled over 500,000 diabetic patients and equal controls matched by age and gender, revealed significant associations between old age, male gender and increased hazards of amputation.[59] The Seattle diabetic foot study revealed that previous ulcerations and amputations were significant predictors of LEA.[38] Accordingly, history of ulceration was found to be the strongest risk factor significantly associated with amputation in current study, however, history of LEA was not found to be statistically significant.

A retrospective study investigating the predictors of major amputation revealed statistically significant relationship between insulin therapy and higher incidence of major LEA which is consistent with the current study findings.[60] Fasting plasma glucose level was another significant risk factor that was found to be negatively associated with major amputation in current study.[34] In contrast, the literature suggests fasting plasma glucose to be positively associated with minor and major amputations. The contradictory results might be attributable to the small sample size, explaining the negative association between fasting plasma glucose level and major amputation found in the study.

4.2 Study strengths

To our knowledge, the current study marks a first attempt to specifically investigate lower extremity amputation risk factors among patients with diabetic foot ulcer in the Republic of Armenia. Cases and controls underwent their treatment in the same medical centers and were

identified from the same data sources, which increased the confidence that the selected comparison group was a representative sample of the source population which produced the cases. The controls were selected independently of exposure status. The case/control status of the study participants was identified according to the screening procedure results and the provided information on the present illnesses in the medical records, which assured compliance with study eligibility criteria for all study participants. The study sample can be considered representative for Armenia as the medical centers included in the study are the main tertiary care facilities in the country that provide highly specialized health care to diabetic patients, the majority of whom are individuals from remote Armenian provinces. High response rate was another strength of the study, which eliminates the potential effect of non-response bias on the results. Exposure misclassification due to recall bias was minimized as the majority of information on the potential risk factors was obtained from the medical records. However, recall bias may take place as LEA patients are more prone to over report details on their exposure in contrast with non-LEA patients.

4.3 Study limitations

The current study had several limitations inherent to most of the case-control studies. Information on the exposure was a subject to interviewer bias as the student-investigator's knowledge of the case/control status of the participants may affect the way responses are recorded. To reduce the interviewer bias, a standardized wording was designed that must be used for asking the questions on exposure. Social desirability bias in a form of underreporting unhealthy lifestyle behaviors is also likely to occur. Non-coverage bias may take place as some of the medical records were not available or the contact information for some patients provided in the medical records was absent, wrong, unregistered or unreachable. Medical records may

introduce a potential risk in terms of limited accuracy and completeness. Measurement error due to missing laboratory values may reduce the study power and lead to bias in the estimation of the OR resulting in invalid conclusions.

4.4 Recommendations

The results of the current study suggest that implementation of screening programs for identification of at-risk foot would be valuable to patients, health providers and healthcare system in Armenia. Diabetic foot care educational program should be initiated and counseling tailored to patient's education and social background should be offered to patients with at-risk foot. Promotion of the study findings among healthcare providers with a significant role in the disease management is also considered necessary.

In addition, further research is warranted to investigate the epidemiological patterns of diabetic foot ulcer and diabetes-related lower extremity amputations in Armenia. Moreover, prospective studies involving more detailed lifestyle as well as other possible risk factors are recommended for further conclusions.

5. Conclusion

In conclusion, the presented research was the first case-control study conducted in Armenia, investigating the risk factors of lower extremity amputation in patients with diabetic foot ulcer. According to the study findings, gender, age, history of foot ulcer and erythrocyte sedimentation rate was statistically significantly associated with LEA. Also, the risk factors for major LEA in patients with diabetes-related amputations were found to be diabetes treatment and total leukocyte count. Overall, the study resulted in findings consistent with the international literature and in ideas for further research.

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Table 1.1 Socio-demographic and lifestyle characteristics of cases and controls

Characteristics	Major & Minor Amputation Group (n=77)		DFU Group (n=77)
	Major Amputation Group (n=37)	Minor Amputation Group (n=40)	
Demographic			
Gender, n (%)			
Male	23 (62.16%)	26 (65.00%)	36 (46.75%)
Female	14 (37.84%)	14 (35.00%)	41 (53.25%)
Age, years			
Mean (SD)	68.70 (7.76)	64.72 (8.47)	56.27 (10.07)
Education, n (%)			
School (less than 10 years)	6 (16.22%)	6 (15.00%)	7 (9.09%)
School (10 years)	13 (35.14%)	22 (55.00%)	46 (59.74%)
Professional technical (10-13 years)	10 (27.03%)	6 (15.00%)	13 (16.88%)
Institute/University	7 (18.92%)	6 (15.00%)	11 (14.29%)
Post-graduate	1 (2.70%)	0 (0.00%)	0 (0.00%)
Employment, n (%)			
Employed	12 (32.43%)	20 (50.00%)	24 (31.17%)
Unemployed	3 (8.11%)	5 (12.50%)	17 (22.08%)
Retired	22 (59.46%)	15 (37.50%)	36 (46.75%)
Marital status, n (%)			
Married	33 (89.19%)	39 (97.50%)	65 (84.42%)
Single	0 (0.00%)	0 (0.00%)	4 (5.19%)
Widowed	3 (8.11%)	0 (0.00%)	7 (9.09%)
Divorced/Separated	1 (2.70%)	1 (2.50%)	1 (1.30%)
Body mass index			
Mean (SD)	28.37 (4.53)	30.53 (4.32)	31.03 (6.34)

Household monthly expenses, n (%)			
Less than 50.000 drams	7 (18.92%)	7 (17.50%)	17 (22.08%)
From 50,000 – 100,000 drams	13 (35.14%)	13 (32.50%)	41 (53.25%)
From 100,001 – 200,000 drams	6 (16.22%)	10 (25.00%)	11 (14.29%)
From 200,001 – 300,000 drams	0 (0.00%)	2 (5.00%)	5 (6.49%)
Above 300,000 drams	2 (5.41%)	1 (2.50%)	0 (0.00%)
Don't know	7 (18.92%)	4 (10.00%)	2 (2.60%)
Refuse to answer	2 (5.41%)	3 (7.50%)	1 (1.30%)
Residency, n (%)			
Yerevan	16 (43.24%)	9 (22.50%)	16 (20.78%)
Aragatsotn	2 (5.41%)	1 (2.50%)	12 (15.58%)
Ararat	1 (2.70%)	6 (15.00%)	4 (5.19%)
Armavir	2 (5.41%)	3 (7.50%)	6 (7.79%)
Gegharkunik	2 (5.41%)	6 (15.00%)	11 (14.29%)
Kotayk	6 (16.22%)	4 (10.00%)	11 (14.29%)
Lori	1 (2.70%)	4 (10.00%)	5 (6.49%)
Shirak	2 (5.41%)	1 (2.50%)	3 (3.90%)
Syunik	2 (5.41%)	1 (2.50%)	1 (1.30%)
Tavush	3 (8.11%)	2 (5.00%)	6 (7.79%)
Vayots Dzor	0 (0.00%)	3 (7.50%)	2 (2.60%)
Lifestyle			
Ever smoking, n (%)			
Yes	21 (56.76%)	21 (52.50%)	31 (40.26%)
No	16 (43.24%)	19 (47.50%)	46 (59.74%)
Current smoking status, n (%)			
Yes	7 (18.92%)	6 (15.00%)	18 (23.38%)
No	14 (37.84%)	15 (37.50%)	13 (16.88%)
N/A	16 (43.24%)	19 (47.50%)	46 (59.74%)
Number of cigarettes			
Mean (SD)	18.42 (12.56)	20.00 (9.48)	21.72 (14.14)
Smoking years			
Mean (SD)	32.90 (13.98)	27.61 (14.74)	21.41 (13.64)
Smoking status before hospitalization, n (%)			
Yes	12 (32.43%)	14 (35.00%)	19 (24.68%)
No	9 (24.32%)	7 (17.50%)	12 (15.58%)
N/A	16 (43.24%)	19 (47.50%)	46 (59.74%)
Number of cigarettes before hospitalization			
Mean (SD)	30.33 (20.01)	26.00 (17.32)	24.78 (22.73)

Alcohol use frequency, n (%)			
Never	18 (48.65%)	16 (40.00%)	44 (57.14%)
Less than 1 drink a week	10 (27.03%)	17 (42.50%)	26 (33.77%)
1-3 drinks a week	4 (10.81%)	3 (7.50%)	6 (7.79%)
4-6 drinks a week	5 (13.51%)	3 (7.50%)	1 (1.30%)
7-13 drinks a week	0 (0.00%)	1 (2.50%)	0 (0.00%)
Alcohol use before hospitalization, n (%)			
Yes	13 (35.14%)	21 (52.50%)	30 (38.96%)
No	6 (16.22%)	3 (7.50%)	3 (3.90%)
N/A	18 (48.65%)	16 (40.00%)	44 (57.14%)
Alcohol use frequency before hospitalization, n (%)			
Less than 1 drink a week	8 (22.86%)	12 (30.00%)	20 (25.97%)
1-3 drinks a week	1 (2.86%)	7 (17.50%)	10 (12.99%)
4-6 drinks a week	4 (11.43%)	2 (5.00%)	0 (0.00%)
7-13 drinks a week	0 (0.00%)	1 (2.50%)	0 (0.00%)
N/A	22 (62.86%)	18 (45.00%)	47 (61.04%)
Having a glucometer, n (%)			
Yes	29 (78.38%)	35 (87.50%)	62 (80.52%)
No	8 (21.62%)	5 (12.50%)	15 (19.48%)
Glucometer use frequency			
Mean (SD)	0.80 (1.09)	0.81 (0.82)	1.01 (0.99)
Shoes			
Trainers, lace-ups, boots (low heel), extra depth/surgical shoes	4 (10.81%)	6 (15.00%)	17 (22.08%)
Casual shoes, bar or buckle fastened shoes, slippers	22 (59.46%)	21 (52.50%)	46 (59.74%)
Open-toe sandals, high-heeled shoes, flip-flops	11 (29.73%)	13 (32.50%)	14 (18.18%)
Family history			
History of DFU in relatives, n (%)			
Yes	8 (21.62%)	18 (45.00%)	29 (37.66%)
No	28 (75.68%)	21 (52.50%)	47 (61.04%)
Don’t know	1 (2.70%)	1 (2.50%)	1 (1.30%)
History of LEA in relatives, n (%)			
Yes	2 (5.41%)	8 (20.00%)	8 (10.39%)
No	35 (94.59%)	32 (80.00%)	67 (87.01%)
Don’t know	0 (0.00%)	0 (0.00%)	2 (2.60%)

Table 1.2 Clinical and laboratory characteristics of cases and controls

Characteristics	Major & Minor Amputation Group (n=77)		DFU Group (n=77)
	Major Amputation Group (n=37)	Minor Amputation Group (n=40)	
Clinical			
DM type, n (%)			
Type 1 DM	0 (0.00%)	0 (0.00%)	7 (9.09%)
Type 2 DM	37 (100%)	40 (100%)	70 (90.91%)
DM duration [1]			
Mean (SD)	16.63 (7.78)	16.18 (9.23)	11.70 (6.77)
DM treatment, n (%)			
Diet	8 (21.62%)	1 (2.50%)	4 (5.19%)
Diet + Oral agents	5 (13.51%)	8 (20.00%)	23 (29.87%)
Diet + Insulin	21 (56.76%)	25 (62.50%)	35 (45.45%)
Diet + Insulin + oral	3 (8.11%)	6 (15.00%)	15 (19.48%)
History of DFU, n (%)			
Yes	26 (70.27%)	20 (50.00%)	2 (2.60%)
No	11 (29.73%)	20 (50.00%)	75 (97.40%)
History of LEA, n (%)			
None	20 (54.05%)	28 (70.00%)	77 (100%)
Surgical debridement	4 (10.81%)	1 (2.5%)	0 (0.00%)
Minor amputation	7 (18.92%)	10 (25.00%)	0 (0.00%)
Major amputation	6 (16.22%)	1 (2.50%)	0 (0.00%)
Hypertension, n (%)			
Yes	28 (75.68%)	23 (57.50%)	59 (76.62%)
No	9 (24.32%)	17 (42.50%)	18 (23.38%)
Arrhythmia, n (%)			
Yes	9 (24.32%)	5 (12.50%)	8 (10.39%)
No	28 (75.68%)	35 (87.50%)	69 (89.61%)
Ischemic heart disease, n (%)			
Yes	22 (59.46%)	14 (35.00%)	38 (49.35%)
No	15 (40.54%)	26 (65.00%)	39 (50.65%)

Stable angina, n (%)			
Yes	1 (2.70%)	2 (5.00%)	6 (7.79%)
No	36 (97.30%)	38 (95.00%)	71 (92.21%)
Myocardial infarction, n (%)			
Yes	6 (16.22%)	6 (15.00%)	7 (9.09%)
No	31 (83.78%)	34 (85.00%)	70 (90.91%)
Cardiosclerosis, n (%)			
Yes	13 (35.14%)	7 (17.50%)	27 (35.06%)
No	24 (64.86%)	33 (82.50%)	50 (64.94%)
Hypertrophic cardiomyopathy, n (%)			
Yes	9 (24.32%)	6 (15.00%)	12 (15.58%)
No	28 (75.68%)	34 (85.00%)	65 (84.42%)
Heart valve disease, n (%)			
Yes	1 (2.70%)	0 (0.00%)	3 (3.90%)
No	36 (97.30%)	40 (100%)	74 (96.10%)
Pulmonary hypertension, n (%)			
Yes	2 (5.41%)	2 (5.00%)	1 (1.30%)
No	35 (94.59%)	38 (95.00%)	76 (98.70%)
Stroke, n (%)			
Yes	2 (5.41%)	2 (5.00%)	1 (1.30%)
No	35 (94.59%)	38 (95.00%)	76 (98.70%)
Flatfoot			
Yes	2 (5.41%)	4 (10.00%)	4 (5.19%)
No	35 (94.59%)	36 (90.00%)	73 (94.81%)
Ulcer characteristics			
Ulcer Depth, n (%)			
Dermis	0 (0.00%)	0 (0.00%)	57 (74.03%)
Subcutaneous tissue	0 (0.00%)	0 (0.00%)	18 (23.38%)
Joint	0 (0.00%)	0 (0.00%)	2 (2.60%)
Bone	37 (100%)	40 (100%)	0 (0.00%)
Ulcer Location, n (%)			
Dorsal	0 (0.00%)	3 (7.50%)	3 (3.90%)
Plantar	0 (0.00%)	1 (2.50%)	3 (3.90%)
Border	0 (0.00%)	36 (90.00%)	71 (92.21%)
N/A	37 (100%)	0 (0.00%)	0 (0.00%)
Ulcer Level, n (%)			
Forefoot	0 (0.00%)	38 (95.00%)	73 (94.81%)
Midfoot	0 (0.00%)	1 (2.50%)	0 (0.00%)
Hindfoot	0 (0.00%)	1 (2.50%)	4 (5.19%)
Above the ankle	37 (100%)	0 (0.00%)	0 (0.00%)

Infection, n (%)			
Yes	37 (100%)	40 (100%)	14 (18.18%)
No	0 (0.00%)	0 (0.00%)	63 (81.82%)
Abscess/Flegmona, n (%)			
Yes	37 (100%)	40 (100%)	3 (3.90%)
No	0 (0.00%)	0 (0.00%)	74 (96.10%)
Osteomyelitis, n (%)			
Yes	37 (100%)	40 (100%)	0 (0.00%)
No	0 (0.00%)	0 (0.00%)	77 (100%)
Laboratory Parameters			
Hemoglobin [2]			
Mean (SD)	121.97 (21.67)	124.92 (19.31)	134.61 (17.92)
Total Leukocyte Count [3]			
Mean (SD)	13.64 (6.77)	10.23 (4.19)	7.05 (2.63)
Erythrocyte Sedimentation Rate [4]			
Mean (SD)	49.8 (19.07)	48.71 (19.16)	28.97 (18.20)
Fibrinogen [5]			
Mean (SD)	482.12 (183.48)	470.56 (97.39)	323.59 (86.83)
Total Protein [6]			
Mean (SD)	94.64 (135.42)	74.01 (6.49)	75.53 (7.24)
Fasting Plasma Glucose [7]			
Mean (SD)	11.90 (5.43)	14.52 (5.20)	12.81 (4.85)
Blood Urea Nitrogen [8]			
Mean (SD)	9.17 (4.25)	7.86 (4.64)	7.32 (4.23)
Serum Creatinine [9]			
Mean (SD)	109.46 (57.59)	91.30 (34.83)	95.75 (76.23)
Urine PH [10]			
Mean (SD)	4.85 (0.54)	5.01 (0.63)	4.87 (0.65)
Urine Protein, n (%) [11]			
Yes	20 (90.91%)	20 (80.00%)	41 (53.25%)
No	2 (9.09%)	5 (20.00%)	36 (46.75%)
Urine Glucose, n (%) [12]			
Yes	4 (44.44%)	8 (50.00%)	49 (63.64%)
No	5 (55.56%)	8 (50.00%)	28 (36.36%)
Ketone Bodies in Urine, n (%) [13]			
Yes	4 (40.00%)	5 (29.41%)	12 (15.79%)
No	6 (60.00%)	12 (70.59%)	64 (84.21%)

Note: Missing values were excluded before calculating the percentages for all variables.

([1]) DM duration had no data value for 33 patients (2 missing in DEBR/non-surgical patients, 13 missing in minor LEA patients and 18 missing in major LEA patients).

([2]) Hemoglobin had no data value for 2 patients (2 missing in minor LEA patients)
([3]) Total leukocyte count had no data value for 2 patients (2 missing in minor LEA patients)
([4]) Erythrocyte sedimentation rate had no data value for 6 patients (1 missing in DEBR/non-surgical patients, 3 missing in minor LEA patients and 2 missing in major LEA patients)
([5]) Fibrinogen had no data value for 39 patients (18 missing for DEBR/non-surgical treatment patients, 15 missing for minor LEA patients and 6 missing for major LEA patients)
([6]) Total protein had no data value for 43 patients (21 missing for DEBR/non-surgical treatment patients, 14 missing for minor LEA patients and 8 missing for major LEA patients)
([7]) Fasting plasma glucose had no data value for 3 patients (1 missing for DEBR/non-surgical treatment patients, 1 missing for minor LEA patients and 1 missing for major LEA patients)
([8]) Blood urea nitrogen had no data value for 14 patients (6 missing for DEBR/non-surgical treatment patients, 5 missing for minor LEA patients and 3 missing for major LEA patients)
([9]) Serum Creatinine had no data value for 2 patients (2 missing for DEBR/non-surgical treatment patients)
([10]) Urine PH had no data value for 24 patients (14 missing for minor LEA patients and 11 missing for major LEA patients)
([11]) Urine protein had no data value for 30 patients (15 missing for minor LEA patients and 15 missing for major LEA patients)
([12]) Urine glucose had no data value for 52 patients (24 missing for minor LEA patients and 28 missing for major LEA patients)
([13]) Ketone bodies had no data value for 51 patients (1 missing for DEBR/non-surgical treatment patients, 23 missing for minor LEA patients and 27 missing for major LEA patients)

Table 2.1 Simple logistic regression analysis of socio-demographic and lifestyle risk factors associated with LEA in patients with diabetic foot ulcer

Characteristics	(OR)	(95% CI)	p-value
Socio-demographic and Lifestyle			
Gender			
Female	1.00		
Male	1.99	1.04-3.79	**0.0347**
Age, years			**<0.0001**
<50	1.00		
51-60	4.25	0.87-20.70	0.073
61-70	13.30	2.80-63.04	0.001
>70	39.1	6.75-226.06	<0.0001
Education			0.3205
School (less than 10 years)	1.00		
School (10 years)	0.44	0.15-1.24	0.122
Professional technical (10-13 years)	0.71	0.21-2.34	0.584
Institute/University/Post-graduate	0.74	0.21-2.51	0.633
Employment			0.1068
Employed	1.00		
Unemployed	0.35	0.13-0.95	0.040
Retired	0.77	0.38-1.55	0.466
Marital status			0.0681
Married	1.00		
Widowed Single/Divorced/Separated	0.37	0.12-1.12	0.080
Body mass index	0.95	0.89-1.00	0.0834
Residency			0.3931
Yerevan	1.00		
Aragatsotn	0.16	0.03-0.65	0.011
Ararat	1.12	0.28-4.44	0.872
Armavir	0.53	0.13-2.04	0.359
Gegharkunik	0.46	0.15-1.40	0.175
Kotayk	0.58	0.20-1.68	0.317
Lori	0.64	0.15-2.56	0.529
Shirak	0.64	0.11-3.57	0.611
Syunik	1.92	0.18-20.10	0.586
Tavush	0.53	0.13-2.04	0.359
Vayots Dzor	0.96	0.14-6.39	0.966

Ever smoking			
No	1.00		
Yes	1.78	0.93-3.37	0.0754
Current smoking status			**0.0138**
No	1.00		
Yes	0.32	0.12-1.85	0.022
N/A	0.34	0.15-0.75	0.007
Number of cigarettes	0.98	0.94-1.01	0.2494
Smoking years	1.03	1.00-1.05	**0.0028**
Smoking status before hospitalization			0.2054
No	1.00		
Yes	1.02	0.39-2.66	0.957
N/A	0.57	0.23-1.35	0.205
Number of cigarettes before hospitalization	1.01	0.99-1.03	0.1968
Alcohol use frequency			**0.0329**
Never	1.00		
Less than 1 drink a week	1.34	0.66-2.70	0.408
1-3 drinks a week	1.50	0.46-4.90	0.493
4-13 drinks a week	11.64	1.40-96.44	0.023
Alcohol use before hospitalization			0.0966
No	1.00		
Yes	0.37	0.09-1.52	0.172
N/A	0.25	0.06-1.02	0.054
Alcohol use frequency before hospitalization			0.4620
Less than 1 drink a week	1.00		
1-13 drinks a week	1.5	0.54-4.12	0.432
N/A	0.85	0.40-1.80	0.673
Having a glucometer			
No	1.00		
Yes	1.25	0.54-2.80	0.5966
Glucometer use frequency	0.86	0.62-1.22	0.4103
Shoes			0.1003
Trainers, lace-ups, boots (low heel), extra depth/surgical shoes	1.00		
Casual shoes, bar or buckle fastened shoes, slippers	1.58	0.61-1.21	0.305
Open-toe sandals, high-heeled shoes, flip-flops	2.91	1.04-8.09	0.040

Family history

History of DFU in relatives			0.7614
No	1.00		
Yes	0.85	0.44-1.66	0.656
Don’t know	1.91	0.16-21.86	0.600
History of LEA in relatives			
No			
Yes	1.25	0.46-3.36	0.6577

Table 2.2 Simple logistic regression analysis of clinical and laboratory risk factors associated with LEA in patients with diabetic foot ulcer

Characteristics	(OR)	(95% CI)	p-value
Clinical and Laboratory			
DM treatment			0.0503
Diet	1.00		
Diet + Oral agents	0.25	0.06-0.97	0.046
Diet + Insulin	0.58	0.16-2.05	0.402
Diet + Insulin + oral	0.26	0.06-1.12	0.072
History of DFU			
No	1.00		
Yes	55.64	12.71-243.53	**<0.0001**
Hypertension			
No	1.00		
Yes	0.62	0.30-1.26	0.1896
Arrhythmia			
No	1.00		
Yes	1.91	0.75-4.87	0.1647
Ischemic heart disease			
No	1.00		
Yes	0.90	0.47-1.69	0.7470
Stable angina			
No	1.00		
Yes	0.47	0.11-1.99	0.2984
Myocardial infarction			
No	1.00		
Yes	1.84	0.68-4.97	0.2181
Cardiosclerosis			
No	1.00		
Yes	0.64	0.32-1.29	0.2199
Hypertrophic cardiomyopathy			
No	1.00		
Yes	1.31	0.56-3.02	0.5246
Heart valve disease			
No	1.00		
Yes	0.32	0.03-3.19	0.3002

Pulmonary hypertension			
No	1.00		
Yes	4.16	0.45-38.14	0.1586
Stroke			
No	1.00		
Yes	4.16	0.45-38.14	0.1586
Flatfoot			
No	1.00		
Yes	1.54	0.41-5.69	0.5117
Hemoglobin	0.96	0.95-0.98	**0.0004**
Total Leukocyte Count	1.38	1.21-1.58	**<0.0001**
Erythrocyte Sedimentation Rate	1.05	1.03-1.07	**<0.0001**
Fasting Plasma Glucose	1.01	0.95-1.08	0.5879
Blood Urea Nitrogen	1.06	0.98-1.15	0.1025
Serum Creatinine	1.00	0.99-1.00	0.6747

Table 3.1 Simple logistic regression analysis of socio-demographic and lifestyle risk factors associated with major LEA in patients with diabetic foot amputation

Characteristics	(OR)	(95% CI)	p-value
Socio-demographic and Lifestyle			
Gender			
Female	1.00		
Male	0.88	0.34-2.24	0.7959
Age, years			**0.0433**
<60	1.00		
61-70	3.63	1.06-12.40	0.039
>70	2.38	0.63-8.89	0.196
Education			0.3181
School (less than 10 years)	1.00		
School (10 years)	0.59	0.15-2.21	0.436
Professional technical (10-13 years)	1.66	0.36-0.60	0.510
Institute/University/Post-graduate	1.33	0.28-6.27	0.716
Employment			0.1540
Employed	1.00		
Unemployed	1	0.20-4.95	1.000
Retired	2.44	0.92-6.45	0.071
Marital status			
Married	1.00		
Widowed/Single/Divorced/Separated	4.72	0.50-44.39	0.1285
Body mass index	0.89	0.80-0.99	**0.0334**
Residency			0.2252
Yerevan	1.00		
Aragatsotn	1.12	0.08-0.20	0.927
Ararat	0.09	0.009-0.90	0.041
Armavir	0.37	0.05-2.68	0.328
Gegharkunik	0.18	0.03-1.13	0.068
Kotayk	0.84	0.18-3.80	0.825
Lori	0.14	0.01-1.45	0.100
Shirak	1.12	0.08-14.20	0.927
Syunik	1.12	0.08-14.20	0.927
Tavush	0.84	0.11-6.03	0.866
Ever smoking			
No	1.00		
Yes	1.18	0.48-2.91	0.7077

Current smoking status			0.8816
No	1.00		
Yes	1.25	0.33-4.63	0.739
N/A	0.90	0.33-2.41	0.838
Number of cigarettes	1.00	0.95-1.06	0.7985
Smoking years	1.01	0.98-1.03	0.3189
Smoking status before hospitalization			0.7614
No	1.00		
Yes	0.66	0.19-2.33	0.526
N/A	0.65	0.19-2.15	0.486
Number of cigarettes before hospitalization	1.00	0.97-1.02	0.8481
Alcohol use frequency			0.5537
Never	1.00		
Less than 1 drink a week	0.52	0.18-1.46	0.218
1-3 drinks a week	1.18	0.22-6.11	0.839
4-13 drinks a week	1.11	0.25-4.86	0.839
Alcohol use before hospitalization			0.2322
No	1.00		
Yes	0.30	0.06-1.45	0.138
N/A	0.56	0.12-2.62	0.464
Alcohol use frequency before hospitalization			0.2764
Less than 1 drink a week	1.00		
1-13 drinks a week	0.75	0.18-3.03	0.687
N/A	1.83	0.61-5.45	0.276
Having a glucometer			
No	1.00		
Yes	0.51	0.15-1.75	0.2846
Glucometer use frequency	0.89	0.54-1.47	0.6650
Shoes			0.7882
Trainers, lace-ups, boots (low heel), extra depth/surgical shoes	1.00		
Casual shoes, bar or buckle fastened shoes, slippers	1.57	0.38-6.36	0.527
Open-toe sandals, high-heeled shoes, flip-flops	1.26	0.28-5.67	0.755

Family history

History of DFU in relatives			
No	1.00		
Yes	0.99	0.96-1.03	0.9861
History of LEA in relatives			
No	1.00		
Yes	0.22	0.04-1.15	**0.0491**

Table 3.2 Simple logistic regression analysis of clinical and laboratory risk factors associated with major LEA in patients with diabetic foot amputation

Characteristics	(OR)	(95% CI)	p-value
Clinical and Laboratory			
DM treatment			**0.0431**
Diet	1.00		
Diet + Oral agents	0.07	0.007-0.82	0.034
Diet + Insulin	0.10	0.01-0.90	0.041
Diet + Insulin + oral	0.06	0.005-0.76	0.030
History of DFU			
No	1.00		
Yes	2.36	0.92-6.04	0.0685
History of LEA			
No	1.00		
Yes	1.38	0.90-2.12	0.1275
Hypertension			
No	1.00		
Yes	2.16	0.80-5.79	0.1194
Arrhythmia			
No	1.00		
Yes	2.25	0.67-7.47	0.1771
Ischemic heart disease			
No	1.00		
Yes	2.72	1.08-6.85	**0.0308**
Stable angina			
No	1.00		
Yes	0.52	0.04-6.07	0.5987
Myocardial infarction			
No	1.00		
Yes	1.09	0.31-3.75	0.8832
Cardiosclerosis			
No	1.00		
Yes	2.55	0.88-7.36	0.0765
Hypertrophic cardiomyopathy			
No	1.00		
Yes	1.82	0.57-5.73	0.3012

Pulmonary hypertension			
No	1.00		
Yes	1.08	0.14-8.12	0.9362
Stroke			
No	1.00		
Yes	1.08	0.14-8.12	0.9362
Flatfoot			
No	1.00		
Yes	0.51	0.08-2.98	0.4476
Hemoglobin	0.99	0.97-1.01	0.5288
Total Leukocyte Count	1.12	1.02-1.22	**0.0089**
Erythrocyte Sedimentation Rate	1.00	0.97-1.02	0.8065
Fasting Plasma Glucose	0.90	0.82-0.99	**0.0316**
Blood Urea Nitrogen	1.07	0.95-1.20	0.2148
Serum Creatinine	1.00	0.99-1.01	0.0865

Table 4 Multiple logistic regression analysis of risk factors associated with LEA in patients with diabetic foot ulcer

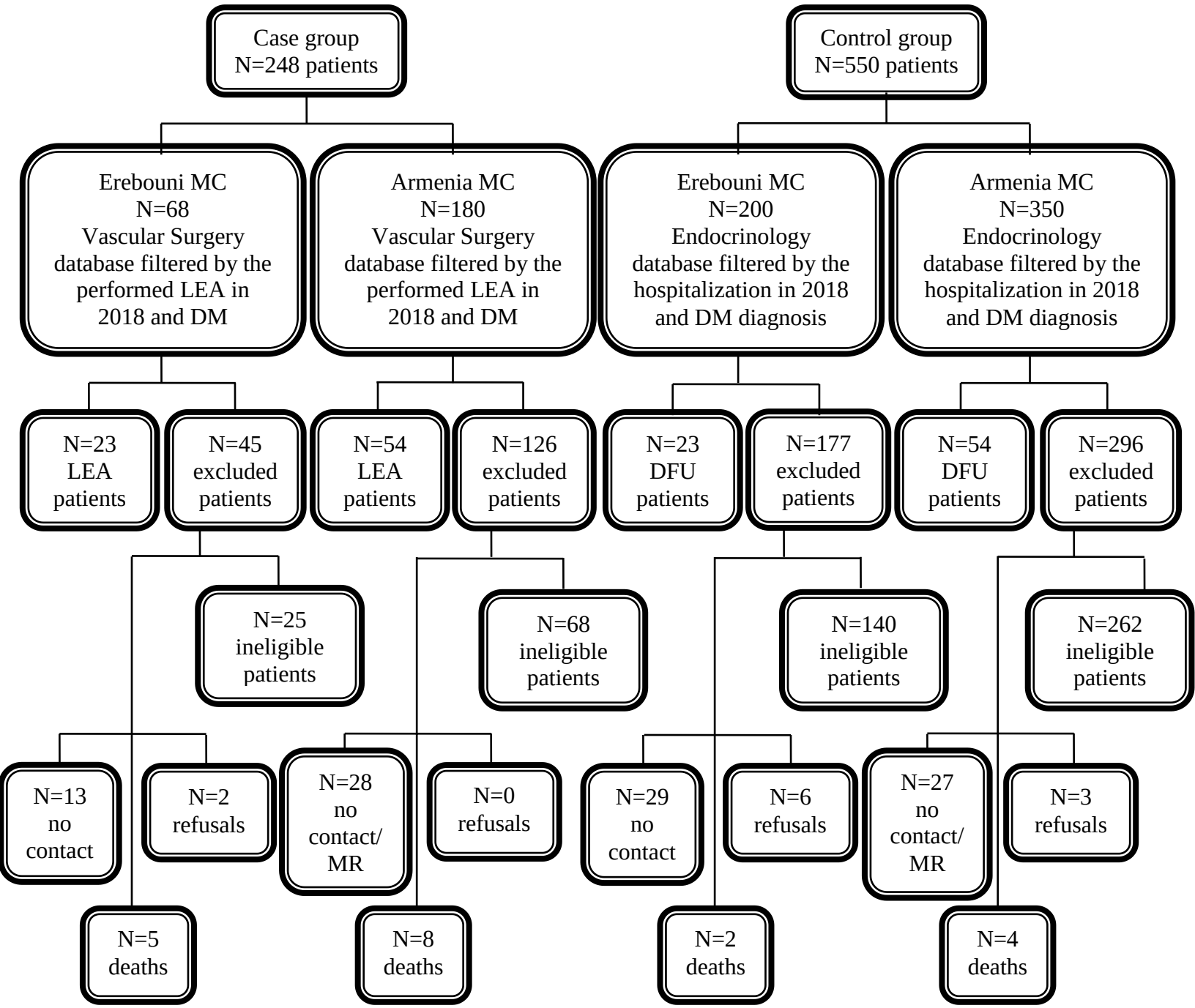
Characteristics	(OR)	(95% CI)	p-value
Age, years			
<50	1.00		
51-60	8.16	0.69-95.42	0.094
61-70	17.86	1.57-202.28	**0.020**
>70	68.58	5.08-924.66	**0.001**
History of DFU			
No	1.00		
Yes	123.24	13.15-1154.65	**<0.0001**
Total Leukocyte Count	1.37	1.15-1.64	**<0.0001**

Table 5 Multiple logistic regression analysis of risk factors associated with major LEA in patients with diabetic foot amputation

Characteristics	(OR)	(95% CI)	p-value
DM treatment			
Diet	1.00		
Diet + Oral agents	0.07	0.004-1.38	0.082
Diet + Insulin	0.07	0.004-1.09	0.058
Diet + Insulin + oral	0.02	0.0009-0.48	**0.016**
Total Leukocyte Count	1.19	1.05-1.35	**0.006**
Fasting Plasma Glucose	0.85	0.76-0.96	**0.010**

Figure

Flow chart outlining the study sample selection in Erebouni Medical Center and Armenia Medical Center



Appendix 1 Summary of prevalence rates and odds ratios/risk ratios of different risk factors for lower extremity amputation from the literature

Risk factor	Prevalence, (%)		OR	RR	References
	In LEA Group	In DFU Group			
Gender					
Male	52.4	35.6	3.81		**(40)**
Age					
>70 years	0.9			0.34	**(38)**
DM treatment					
Insulin therapy	90.5	36.1	10.95		**(40)**
History of LEA	28.6	0.8	16.58		**(40)**
Comorbidities					
Hypertension	96.9	75.0	0.09		**(41)**
Ischemic heart disease	40.0	20.2	2.63		**(40)**
Myocardial infarction	15.0	6.1	2.74		**(40)**
Abscess/Flegmona	41.6		1.67		**(37)**

Appendix 2 Sample size calculation

$$n = \frac{(r+1)}{r} \times \frac{(\bar{p})(1-\bar{p})(Z_\beta + Z_{\alpha/2})^2}{(p_1 - p_2)^2}$$

- For $power = 80\%$, $Z_\beta = 0.8$
- For $\alpha = 0.05$, $Z_{\alpha/2} = 1.96$
- $r = 1.0$, ratio of controls to cases
- The proportion of exposed in the control group: $P_{cont.exp} = 0.4$
- The proportion of cases exposed:

  $P_{case\ exp} = \frac{OR \times P_{cont.exp}}{P_{cont.exp} \times (OR-1)+1} = \frac{2.5 \times 0.4}{0.4 \times (2.5-1)+1} = 0.625$

- The average proportion of exposed:

  $\bar{p} = \frac{0.625 + 0{,}4}{2} = 0.5125$

$$n = \frac{(r+1)}{r} \times \frac{(\bar{p})(1-\bar{p})\left(Z_\beta + Z_{\alpha/2}\right)^2}{(p_1 - p_2)^2}$$

$$n = \frac{(1+1)}{1} \times \frac{(0.5125)(1-0.5125)(0.8+1.96)^2}{(0.625-0.4)^2}$$

$n \approx 76.2816 \approx 77$ (participants in each group)

Therefore, $N = 2 \times 77 = 154$ (participants in total)

Appendix 3 Questionnaire (English version)

Patient ID** __ / __ __ __ Clinic ID* _____ Date (day/month/year) ___/___/_____

Questionnaire

Screening Questions

1. Have you been diagnosed with any of the following diseases in 2018? a) Chronic venous insufficiency, varicose veins b) Acute peripheral artery thrombosis c) Lymphedema d) Congestive heart failure e) Rheumatological diseases f) Malignant neoplasm g) Radiotherapy and immunosuppressive therapy h) Ulcers and amputations due to other reasons than DM	1. ☐ Yes – thank the participant and terminate the interview 0. ☐ No – continue the interview
2. Have you ever been diagnosed with diabetic foot ulcer?	1. ☐ Yes – continue the interview 0. ☐ No – thank the participant and finish the interview
3. What type of treatment have you received in 2018?	2. ☐ Lower extremity amputation _____ (specify) – assign the participant to case group 1. ☐ Surgical debridement/non-surgical treatment – assign to control group

Patient ID** __ / __ __ __ Clinic ID* _____

I. Family History

4. Have one of your close relatives (mother, father, sisters, brothers, daughters, sons, uncles, aunts, nephews, nieces, grandparents, grandchildren, half-siblings, and double cousins) ever been diagnosed with diabetic foot ulcer?	1. ☐ Yes 0. ☐ No 88. ☐ Don't know
5. Have one of your close relatives ever undergone lower extremity amputation?	1. ☐ Yes 0. ☐ No 88. ☐ Don't know

II. Lifestyle

6. Have you ever smoked cigarettes?	1. ☐ Yes 0. ☐ No → **(Go to Q12)**
7. Do you currently smoke cigarettes?	1. ☐ Yes 0. ☐ No → **(Go to Q9)**
8. On average, how many cigarettes per day do you smoke?	_____ (cigarettes)
9. How long have you been smoking?	_____ (years)
10. Were you smoking before the hospitalization?	1. ☐ Yes 0. ☐ No → **(Go to Q12)**
11. On average, how many cigarettes per day were you smoking before the hospitalization?	_____ (cigarettes)

12. On average, how often do you use alcohol containing drinks?	0. ☐ Never → **(Go to Q15)** 1. ☐ Less than 1 drink a week 2. ☐ 1-3 drinks a week 3. ☐ 4-6 drinks a week 4. ☐ 7-13 drinks a week 5. ☐ 14 drinks or more a week
13. Were you using alcohol containing drinks before the hospitalization?	1. ☐ Yes 0. ☐ No → **(Go to Q15)**
14. On average, how often were you using alcohol containing drinks before the hospitalization?	0. ☐ Less than 1 drink a week 1. ☐ 1-3 drinks a week 2. ☐ 4-6 drinks a week 3. ☐ 7-13 drinks a week 4. ☐ 14 drinks or more a week

III. Additional questions

15. Did you have a glucometer (a medical device for checking blood sugar levels) before the hospitalization?	1. ☐ Yes 0. ☐ No → **(Go to Q17)**
16. On average, how many times a day were you checking your blood sugar level with your glucometer before the hospitalization?	_____
17. Have you ever been diagnosed with flatfoot?	1. ☐ Yes 0. ☐ No
18. Which type of shoes were you wearing before the hospitalization?	0. ☐ trainers, lace-ups, boots (low heel), extra depth/surgical shoes 1. ☐ casual shoes, bar or buckle fastened shoes, slippers 2. ☐ open-toe sandals, high-heeled shoes, flip-flops

Patient ID** __ / __ __ __ Clinic ID* _____

IV. Socio-demographic and Anthropometric Characteristics

19. From what part of Armenia you are?	0. ☐ Yerevan 1. ☐ Aragatsotn 2. ☐ Ararat 3. ☐ Armavir 4. ☐ Gegharkunik 5. ☐ Kotayk 6. ☐ Lori 7. ☐ Shirak 8. ☐ Syunik 9. ☐ Tavush 10. ☐ Vayots Dzor
20. What is your gender? **(Do not read)**	1. ☐ Male 0. ☐ Female
21. What is your date of birth?	____/____/_____ (day/month/year)
22. Body mass index What was your average weight before the hospitalization? What is your average height?	_____ (kg/m^2) _____ (kg) _____ (cm)
23. What is your completed educational level?	0. ☐ School (less than 10 years) 1. ☐ School (10 years) 2. ☐ Professional technical education (10-13 years) 3. ☐ Institute/University 4. ☐ Post-graduate

Patient ID** __ / __ __ __ Clinic ID* _____

24. What was your employment status before the hospitalization?	0. ☐ Employed 1. ☐ Unemployed 2. ☐ Student 3. ☐ Retired 4. ☐ Other
25. What was your marital status before the hospitalization?	0. ☐ Married 1. ☐ Single 2. ☐ Widowed 3. ☐ Divorced/Separated
26. On average, what were your household expenses per month before the hospitalization?	0. ☐ Less than 50.000 drams 1. ☐ From 50,000 – 100,000 drams 2. ☐ From 100,001 – 200,000 drams 3. ☐ From 200,001 – 300,000 drams 4. ☐ Above 300,000 drams 88. ☐ Don't know 99. ☐ Refuse to answer

Thank you for your time!

Patient ID** __ / __ __ __ Clinic ID* _____

Medical Record Review Form

27. Diabetes type	1. ☐ Type 1 diabetes 0. ☐ Type 2 diabetes
28. Diabetes duration	_____ (years)
29. Diabetes treatment	0. ☐ Diet 1. ☐ Diet + Oral agents 2. ☐ Diet + Insulin 3. ☐ Diet + Insulin + oral
30. History of foot ulcer	1. ☐ Yes 0. ☐ No
31. History of surgical treatment	0. ☐ None 1. ☐ Debridement 2. ☐ Minor amputation 3. ☐ Major amputation
35. Comorbidities	0. ☐ None 1. ☐ Hypertension 2. ☐ Arrhythmia 3. ☐ Ischemic heart disease 4. ☐ Myocardial infarction 5. ☐ Stroke 6. ☐ Other _____ (specify)
36. Ulcer depth	0. ☐ Dermis 1. ☐ Subcutaneous tissue 2. ☐ Joint 3. ☐ Bone

37. Ulcer location	0. ☐ Dorsal 1. ☐ Plantar 2. ☐ Border
38. Ulcer level	0. ☐ Forefoot 1. ☐ Midfoot 2. ☐ Hindfoot 3. ☐ Above the ankle
39. Clinician-diagnosed wound infection	1. ☐ Yes 0. ☐ No
40. Clinician-diagnosed foot abscess/flegmona	1. ☐ Yes 0. ☐ No
41. Clinician-diagnosed osteomyelitis	1. ☐ Yes 0. ☐ No
42. Hemoglobin	_____ (g/L)
43. Total Leukocyte Count	_____ (10^9/L)
44. Erythrocyte Sedimentation Rate	_____ (mm/hr)
45. Fibrinogen	_____ (g/L)
46. Total Protein	_____ (g/L)
47. Fasting Plasma Glucose	_____ (mmol/L)
48. Blood Urea Nitrogen	_____ (mmol/L)

49. Serum Creatinine	_____ (µmol/l)
50. Urine PH	_____
51. Urine Protein	1. ☐ Yes 0. ☐ No
52. Urine Glucose	1. ☐ Yes 0. ☐ No
53. Ketone Bodies in Urine	1. ☐ Yes 0. ☐ No

Appendix 4 Questionnaire (Armenian version)

Պացիենտի ID-ն ** __ / __ __ __ Կլինիկայի ID-ն * _____ Ամսաթիվ (օր/ամիս/տարի) __/__/____

Հարցաթերթիկ

Սկրինինգ հարցեր

1. Դուք ախտորոշվե՞լ եք հետևյալ հիվանդություններից որևէ մեկով 2018 թ.-ին ա) Քրոնիկ երակային անբավարարություն, երակների վարիկոզ հիվանդություն բ) Խորանիստ անոթների սուր թրոմբոզ գ) Լիմֆեդեմա դ) Կանգային սրտային անբավարարություն ե) Ռևմատոլոգիական հիվանդություններ զ) Չարորակ նորագոյացություններ է) Ռադիոթերապիա և իմունոսուպրեսիվ թերապիա ը) Խոցեր և ամպուտացիաներ այլ պատճառներով բացի շաքարային դիաբետը	1. ☐ Այո – շնորհակալություն հայտնել մասնակցին և ավարտել հարցազրույցը 0. ☐ Ոչ – շարունակել հարցազրույցը
2. Դուք երբևէ ախտորոշվե՞լ եք դիաբետիկ ոտնաթաթի խոցով:	1. ☐ Այո – շարունակել հարցազրույցը 0. ☐ Ոչ – շնորհակալություն հայտնել մասնակցին և ավարտել հարցազրույցը
3. Դուք ի՞նչ բուժում եք ստացել 2018 թ.-ին:	2. ☐ Ստորին վերջույթի ամպուտացիա _____ (մանրամասնեք) – նշանակել դեպքերի խմբին 1. ☐ Նեկրէկտոմիա/Կոնսերվատիվ բուժում – նշանակել ստուգիչների խմբին

Պացիենտի ID-ն ** __ / __ __ __ Կլինիկայի ID-ն * _____

I. Ընտանեկան պատմություն

4. Արդյո՞ք Ձեր մերձավոր ազգականներից որևէ մեկը (մայրը, հայրը, քույրերը, եղբայրները, դուստրերը, որդիները, հորեղբայրները, քեռիները, հորաքույրերը, մորաքույրերը, քրոջ կամ եղբոր որդին, քրոջ կամ եղբոր դուստրը, տատիկները և պապերը, թոռները, խորթ քույրերը և/կամ եղբայրները, և զարմիկները) երբևէ ախտորոշվել է դիաբետիկ ոտնաթաթի խոց հիվանդությամբ:	1. ☐ Այո 0. ☐ Ոչ 88. ☐ Չգիտեմ
5. Արդյո՞ք Ձեր մերձավոր ազգականներից որևէ մեկին երբևէ կատարվել է ստորին վերջույթի ամպուտացիա:	1. ☐ Այո 0. ☐ Ոչ 88. ☐ Չգիտեմ

II. Ապրելակերպ

6. Դուք երբևէ ծխախոտ ծխե՞լ եք:	1. ☐ Այո 0. ☐ Ոչ → **(Անցեք Հ12-ին)**
7. Դուք ներկայումս ծխո՞ւմ եք:	1. ☐ Այո 0. ☐ Ոչ → **(Անցեք Հ9-ին)**
8. Միջինում, օրեկան քանի՞ գլանակ եք ծխում:	_____ (ծխախոտի գլանակ)
9. Որքա՞ն ժամանակ եք ծխել:	_____ (տարի)
10. Դուք ծխո՞ւմ էիք մինչև հոսպիտալացումը:	1. ☐ Այո 0. ☐ Ոչ → **(Անցեք Հ12-ին)**

Պացիենտի ID-ն ** __ / __ __ __ Կլինիկայի ID-ն * _____

11. Միջինում, օրեկան քանի՞ գլանակ էիք ծխում մինչև հոսպիտալացումը:	_____ (ծխախոտի գլանակ)
12. Միջինում, որքան հաճախ եք օգտագործում ալկոհոլ պարունակող ըմպելիքներ:	0. ☐ Երբեք → **(Անցեք Հ15-ին)** 1. ☐ 1 ըմպելիքից քիչ մեկ շաբաթում 2. ☐ 1-3 ըմպելիք մեկ շաբաթում 3. ☐ 4-6 ըմպելիք մեկ շաբաթում 4. ☐ 7-13 ըմպելիք մեկ շաբաթում 5. ☐ 14 և ավելի ըմպելիք մեկ շաբաթում
13. Դուք օգտագործո՞ւմ էիք ալկոհոլ պարունակող ըմպելիքներ մինչև հոսպիտալացումը:	1. ☐ Այո 0. ☐ Ոչ → **(Անցեք Հ15-ին)**
14. Միջինում, որքան հաճախ էիք օգտագործում ալկոհոլ պարունակող ըմպելիքներ մինչև հոսպիտալացումը:	0. ☐ 1 ըմպելիքից քիչ մեկ շաբաթում 1. ☐ 1-3 ըմպելիք մեկ շաբաթում 2. ☐ 4-6 ըմպելիք մեկ շաբաթում 3. ☐ 7-13 ըմպելիք մեկ շաբաթում 4. ☐ 14 և ավելի ըմպելիք մեկ շաբաթում

Պացիենտի ID-ն ** __ / __ __ __ Կլինիկայի ID-ն * _____

III. Հավելյալ հարցեր

15. Դուք ունե՞իք գլյուկոմետր (արյան շաքարի մակարդակի ստուգման բժշկական սարք) մինչև հոսպիտալացումը:	1. ☐ Այո 0. ☐ Ոչ → **(Անցեք Հ17-ին)**
16. Միջինում, օրեկան քանի՞ անգամ էիք ստուգում արյան շաքարի մակարդակը Ձեր գլյուկոմետրով մինչև հոսպիտալացումը:	_____
17. Դուք երբևէ ախտորոշվե՞լ եք հարթաթաթությամբ:	1. ☐ Այո 0. ☐ Ոչ
18. Ի՞նչ տեսակի կոշիկ էիք նախընտրում կրել մինչև հոսպիտալացումը:	0. ☐ սպորտային կոշիկներ, դասական կոշիկներ, երկարաճիտք կոշիկներ (ցածրակրունկ), օրթոպեդիկ կոշիկներ 1. ☐ ամենօրյա կոշիկներ, կապիչով ամրացվող կոշիկներ, տնային հողաթափեր 2. ☐ սանդալներ, բարձրակրունկ կոշիկներ, ծովափնյա հողաթափեր

Պացիենտի ID-ն ** __ / __ __ __ Կլինիկայի ID-ն * _____

IV. Սոցիալ-ժողովրդագրական և մարդաչափական բնութագիր

19. Հայաստանի ո՞ր մասից եք:	0. ☐ Երևան 1. ☐ Արագածոտն 2. ☐ Արարատ 3. ☐ Արմավիր 4. ☐ Գեղարքունիք 5. ☐ Կոտայք 6. ☐ Լոռի 7. ☐ Շիրակ 8. ☐ Սյունիք 9. ☐ Տավուշ 10. ☐ Վայոց Ձոր
20. Ձեր սեռը **(Չկարդալ)**	1. ☐ Տղամարդ 0. ☐ Կին
21. Ձեր ծննդյան ամսաթիվը	_____/_____/_____ (օր/ամիս/տարի)
22. Մարմնի զանգվածի ցուցանիշ Ձեր միջին քաշը մինչև հոսպիտալացումը Ձեր միջին հասակը մինչև հոսպիտալացումը	_____ (կգ/մ^2) _____ (կգ) _____ (սմ)
23. Ձեր կրթությունը	0. ☐ Դպրոց (մինչև 10 տարի) 1. ☐ Դպրոց (10 տարի) 2. ☐ Մասնագիտական տեխնիկական կրթություն (10-13 տարի) 3. ☐ Ինստիտուտ/Համալսարան 4. ☐ Հետբուհական

24. Ձեր աշխատանքային կարգավիճակը մինչև հոսպիտալացումը	0. ☐ Աշխատում էի 1. ☐ Չէի աշխատում 2. ☐ Ուսանող էի 3. ☐ Թոշակի էի անցել 4. ☐ Այլ
25. Ձեր ամուսնական կարգավիճակը մինչև հոսպիտալացումը	0. ☐ Ամուսնացած 1. ☐ Չամուսնացած 2. ☐ Այրի 3. ☐ Ամուսնալուծված
26. Միջինում, որքա՞ն էր Ձեր ընտանիքի ամսեկան ծախսերը մինչև հոսպիտալացումը	0. ☐ Մինչև 50.000 դրամ 1. ☐ 50,000 – 100,000 դրամ 2. ☐ 100,001 – 200,000 դրամ 3. ☐ 200,001 – 300,000 դրամ 4. ☐ 300,000 դրամից ավելի 88. ☐ Չգիտեմ 99. ☐ Հրաժարվում եմ պատասխանել

Շնորհակալություն ժամանակ տրամադրելու համար:

Պացիենտի ID-ն ** __ / __ __ __ Կլինիկայի ID-ն * _____

Բժշկական քարտի ձևաթուղթ

27. Դիաբետի տեսակը	1. ☐ Տիպ 1 դիաբետ 0. ☐ Տիպ 2 դիաբետ
28. Դիաբետի տևողությունը	_____ (տարի)
29. Դիաբետի բուժումը	0. ☐ Դիետա 1. ☐ Դիետա + Պերօրալ միջոցներ 2. ☐ Դիետա + Ինսուլին 3. ☐ Դիետա + Ինսուլին + պերօրալ միջոցներ
30. Անամնեզում կրկնվող ոտնաթաթի խոց	1. ☐ Այո 0. ☐ Ոչ
31. Անամնեզում վիրահատական բուժում	0. ☐ Բացակայում է 1. ☐ Նեկրէկտոմիա 2. ☐ Փոքր ծավալի ամպուտացիա 3. ☐ Մեծ ծավալի ամպուտացիա
35. Ուղեկցող հիվանդություններ	0. ☐ Բացակայում է 1. ☐ Հիպերտենզիա 2. ☐ Առիթմիա 3. ☐ Սրտի իշեմիկ հիվանդություն 4. ☐ Սրտամկանի ինֆարկտ 5. ☐ Ինսուլտ 0. ☐ Այլ _____ (մանրամասնեք)
36. Խոցի խորությունը	0. ☐ Մաշկ 1. ☐ Ենթամաշկային ճարպաբջջանք 2. ☐ Հոդ 3. ☐ Ոսկր

Պացիենտի ID-ն ** __ / __ __ __ Կլինիկայի ID-ն * _____

37. Խոցի տեղակայումը	0. ☐ Դորզալ 1. ☐ Պլանտար 2. ☐ Եզրային
38. Խոցի մակարդակը	0. ☐ Առաջային 1. ☐ Միջային 2. ☐ Հետին 3. ☐ Մինչև կոճը
39. Վերքի ինֆեկցվածությունը	1. ☐ Այո 0. ☐ Ոչ
40. Ստորին վերջույթի աբսցես/ֆլեգմոնա	1. ☐ Այո 0. ☐ Ոչ
41. Օստեոմիելիտ	1. ☐ Այո 0. ☐ Ոչ
42. Հեմոգլոբին	_____ (գ/լ)
43. Ընդհանուր լեյկոցիտների քանակ	_____ (10^9/ լ)
44. Էրիտրոցիտների նստեցման արագություն	_____ (մմ/ժ)
45. Ֆիգրինոգեն	_____ (գ/լ)
46. Ընդհանուր սպիտակուց	_____ (գ/լ)
47. Արյան պլազմայի գլյուկոզան քաղցած ժամանակ	_____ (մմոլ/լ)
48. Միզանյութ	_____ (մմոլ/լ)

Պացիենտի ID-ն ** __ / __ __ __ Կլինիկայի ID-ն * _____

49. Արյան պլազմայի կրեատինին	_____ (մմոլ/լ)
50. Մեզի PH	_____
51. Սպիտակուց մեզում	1. ☐ Այո 0. ☐ Ոչ
52. Գլյուկոզ մեզում	1. ☐ Այո 0. ☐ Ոչ
53. Կետոնային մարմիններ մեզում	1. ☐ Այո 0. ☐ Ոչ

Appendix 5 Introduction script (English version)

Introduction script for the interviewer

Hi. My name is Taguhi. I am a graduate student at the Turpanjian School of Public Health at the American University of Armenia and currently working on my thesis project dedicated to the investigation of the risk factors of lower extremity amputation in patients with diabetes. Your phone number was provided by the Endocrinology/Surgery Department of Armenia Republican Medical Center/Erebouni Medical Center. Could I ask a couple of questions to see whether you can participate in this survey? The information provided by you will be confidential.

- In case of a **DOUGHT**

Explain the purpose of the research, mention the value of the provided information and the importance of his/her contribution to the survey and remind that privacy and confidentiality will be maintained. Try to find reasons for the refusal and politely, without persistence, convince to participate.

- In case of **REFUSAL**

Thank the participant for the allocated time and ask the reason for refusal.

- In case of **AGREEMENT**

Thank and check the eligibility of the participant.

- If the participant is **ELIGIBLE** – continue the interview and proceed to the consent form.
- If the participant is **NOT ELIGIBLE** – thank the participant and finish the interview.

Appendix 6 Introduction script (Armenian version)

<u>Ներածություն սցենար հարցազրուցավարի համար</u>

Բարև Ձեզ: Իմ անունը Թագուհի է: Ես Հայաստանի ամերիկյան համալսարանաի Թրփանճեան Հանրային առողջապահության ֆակուլտետի ավարտական կուրսի ուսանող եմ և այժմ աշխատում եմ իմ մագիստրոսական թեզի վրա, որը նվիրված է շաքարային դիաբետով պացիենտների մոտ ստորին վերջույթի ամպուտացիայի ռիսկի գործոնների ուսումնասիրությանը: Ձեր հեռախոսահամարը տրամադրվել է Արմենիա հանրապետական բժշկական կենտրոնի/Էրեբունի բժշկական կենտրոնի Էնդոկրինոլոգիայի/Վիրաբուժության բաժանմունքից: Կարո՞ղ եմ ձեզ մի քանի հարց տալ, որպեսզի հասկանամ արդյոք կարող եք մասնակցել այս հարցմանը: Ձեր կողմից տրամադրված ինֆորմացիան կմնա գաղտնի:

- <u>ԿԱՍԿԱԾԻ</u> դեպքում

Բացատրել հետազոտության նպատակը, նշել տրամադրված ինֆորմացիայի և մասնակցության կարևորությունը հետազոտության համար և հիշեցնել, որ գաղտնիությունը կպահպանվի: Փորձել պարզել մերժելու պատճառները և քաղաքավարի, առանց պարտադրանքի, համոզել մասնակցել:

- <u>ՄԵՐԺՄԱՆ</u> դեպքում

Շնորհակալություն հայտնել մասնակցին տրամադրած ժամանակի համար և պարզել մերժման պատճառը:

- <u>ՀԱՄԱՁԱՅՆՎԵԼՈՒ</u> դեպքում

Շնորհակալություն հայտնել և ստուգել մասնակցի համապատասխանությունը:

- Եթե մասնակիցը <u>ՀԱՄԱՊԱՏԱՍԽԱՆՈՒՄ Է</u> – շարունակել հարցազրույցը և անցնել համաձայնության ձևին

- Եթե մասնակիցը **ՉԻ ՀԱՄԱՊԱՏԱՍԽԱՆՈՒՄ** – շնորհակալություն հայտնել մասնակցին և ավարտել հարցազրույցը

Appendix 7 Oral consent form (English version)

American University of Armenia

Turpanjian School of Public Health

Institutional Review Board #1

Informed consent form

Hi. My name is Taguhi. I am a graduate student at the Turpanjian School of Public Health at the American University of Armenia. We are conducting a study to investigate the risk factors of lower extremity amputation in patients with diabetes, among adult population in Armenia. The research is conducted among 160 patients who received treatment in Armenia Republican Medical Center and Erebouni Medical Center in 2018.

You are invited to participate in this study, as you received treatment at Endocrinology Department of Armenia Republican Medical Center/ Erebouni Medical Center in 2018, from where your contact information was extracted. Your participation in this study will involve only the current telephone interview that will last 5-7 minutes. I would like to ask you to participate in this study to share some additional details about the course of your disease and your lifestyle habits.

Your decision to participate or refuse to participate will not have any undesirable consequences. You may skip any question you prefer not to answer and you may stop the interview any time without any undesirable consequences for you. The participation in the study will not have a negative impact on you.

Your participation is important for the study. There is no direct benefit for the participation, but the information provided by you and obtained from your medical record will contribute to better understanding of the risk factors of lower extremity amputation in patients with diabetes which could lead to improved management, as well as, delay and prevention of the development of this complication in the future.

The information received from you and your medical record is fully confidential and will be used only for study purposes. No identifiable information will appear on the questionnaire and final report. Your contact information will be destroyed immediately after completing the data collection.

If you have any questions regarding this study you can contact the Principal Investigator of this study, Assistant Professor of the Gerald and Patricia Turpanjian School of Public Health, Dr. Vahe Khachadourian at (060) 612570. If you think you have been hurt by participating in the study or feel you have not been treated fairly you can contact the American University of Armenia Human Protections Administrator, Varduhi Hayrumyan at (060) 61 25 61.

Do you agree to participate?

Thank you.

Appendix 8 Oral consent form (Armenian version)

Հայաստանի Ամերիկյան Համալսարան

Թրփանճեան Հանրային Առողջապահության Ֆակուլտետ

Գիտահետազոտական էթիկայի թիվ 1 հանձնաժողով

Իրազեկ համաձայնության ձև

Բարև Ձեզ: Իմ անունը Թագուհի է: Ես Հայաստանի ամերիկյան համալսարանիՀանրային առողջապահության ֆակուլտետի ավարտական կուրսի ուսանող եմ: Մենք իրականացնում ենք հարցում, որի նպատակն է Հայաստանի չափահաս բնակչության շրջանում ուսումնասիրել ստորին վերջույթի ամպուտացիայի ռիսկի գործոնները շաքարային դիաբետով պացիենտների մոտ: Հետազոտությունը իրականացվում է այն 160 պացիենտների շրջանում, ովքեր բուժում են ստացել Արմենիա հանրապետական բժշկական կենտրոնում/Էրեբունի բժշկական կենտրոնում 2018 թվականին.

Դուք հրավիրված եք մասնակցել այս հետազոտությանը, քանի որ բուժում եք ստացել Արմենիա հանրապետական բժշկական կենտրոնի/ Էրեբունի բժշկական կենտրոնի Էնդոկրինոլոգիայի/Վիրաբուժության բաժանմունքում 2018 թվականին, որտեղից և Ձեր կոնտակտային տվյալները վերցվել են: Ձեր մասնակցությունը այս հարցմանը սահմանափակվում է միայն այս հեռախոսային հարցազրույցով, որը կտևի 5-7 րոպե: Ես կխնդրեի Ձեզ մասնակցել այս հետազոտությանը և կիսվել Ձեր հիվանդության ընթացքի և Ձեր ապրելակերպի վերաբերյալ լրացուցիչ տվյալներով:

Հարցմանը մասնակցելու կամ դրանից հրաժարվելու Ձեր որոշումը չի ունենա որևէ անցանկալի հետևանքներ: Դուք կարող եք բաց թողնել ցանկացած հարց, որին գերադասում եք

չպատասկանել և դադարեցնել հարցազրույցը ցանկացած պահի առանց որևէ անցանկալի հետևանքների: Հարցմանը մասնակցելը բացասական հետևանք չի ունենա Ձեզ համար:

Ձեր մասնակցությունը կարևոր է հետազոտության համար: Ձեր մասնակցությունը չի ենթադրում անմիջական շահ Ձեզ համար, բայց Ձեր տրամադրած և բժշկական գրառումներից վերցված տվյալները կօգնեն ավելի լավ հասկանալ 2-րդ տիպի շաքարային դիաբետով պացիենտների մոտ դիաբետիկ ոտնաթաթի խոցոտման ռիսկի գործոնները, որը ապագայում կօգնի բարելավել այս հիվանդության վերահսկումը, ինչպես նաև, հետաձգել և կանխարգել այս բարդության զարգացումը:

Ձեր կողմից տրամադրված և Ձեր բժշկական գրառումներից վերցված տվյալները ամբողջովին գաղտնի են պահվելու և օգտագործվելու են միայն հետազոտության նպատակով: Ձերանձըբացահայտող որևէինֆորմացիա չի նշվելու հարցաթերթիկի և վերջնական զեկույցի վրա: Ձեր կոնտակտային տվյալները կոչնչացվեն անմիջապես տվյալների հավաքագրումից հետո:

Այս հետազոտության վերաբերյալ հարցեր ունենալու դեպքում կարող եք կապ հաստատել հետազոտության ղեկավար, Հայաստանի ամերիկյան համալսարանի Հանրային առողջապահության ֆակուլտետի դոցենտ ՝ Վահե Խաչադուրյանի հետ, հետևյալ հեռախոսահամարով ՝ (060) 612570. Եթե Դուք կարծում եք, որ հետազոտությանը Ձեր մասնակցությունը Ձեզ վնաս է պատճառել կամ Ձեզ լավ չեն վերաբերվել, կարող եք կապ հաստատել Հայաստանի ամերիկյան Համալսարանի էթիկայի հանձնաժողովի համակարգող, Վարդուհի Հայրումյանի հետ, հետևյալ հեռախոսահամարով՝ (060) 61 25 61.

Համաձա՞յն եք մասնակցել:

Շնորհակալություն:

Appendix 9 Journal form (English version)

ID	Name (first, last)	Phone number	Attempt 1	Attempt 2	Attempt 3	Attempt 4
__ / __ __ __						
__ / __ __ __						
__ / __ __ __						
…						

Disposition Codes

1. Complete response (Respondent fully completes the survey)
2. Incomplete response (Respondent refuses to fully complete the survey)
3. Refusal (Respondent refuses to complete the survey) _____ (specify)
4. No answer
5. Busy number
6. Call later
7. Wrong number
8. Not eligible Participant
    a. Non-Armenian speaking
    b. Non-Resident
    c. Age under 18
    d. Chronic venous insufficiency and/or varicose veins
    e. Acute peripheral artery thrombosis
    f. Lymphedema
    g. Congestive heart failure
    h. Rheumatological diseases (vasculitis, scleroderma, systemic lupus erythematosus, etc.)
    i. Malignant neoplasm
    j. Radiotherapy and immunosuppressive therapy
    k. Ulcers and amputations due to other reasons than DM
9. Death
10. Other _____ (specify)

Appendix 10 Journal form (Armenian version)

ID	Անուն Ազգանուն	Phone number	Փորձ 1	Փորձ 2	Փորձ 3	Փորձ 4
__/ __ __ __						
__/ __ __ __						
__/ __ __ __						
…						

Դիրքորոշման կոդեր

1. Ամբողջական պատասխան (Պատասխանողը ամբողջովին ավարտել է հարցումը)
2. Ոչ ամբողջական պատասխան (Պատասխանողը հրաժարվում է ամբողջովին ավարտել հարցումը)
3. Մերժում (Պատասխանողը հրաժարվում է մասնակցել հարցմանը) _____ (մանրամասներ)
4. Պատասխան չկա
5. Զբաղված հեռախոսահամար
6. Զանգահարել ուշ
7. Սխալ հեռախոսահամար
8. Չհամապատասխանող մասնակից
    ա) Հայերենին չտիրապետող անձ
    բ) Ոչ ռեզիդենտ
    գ) Տարիքը 18-ից ցածր
    դ) Քրոնիկ երակայինանբավարարություն և/կամ երակների վարիկոզ հիվանդություն
    ե) Ստորին վերջույթների խորանիստանոթների սուր թրոմբոզ
    զ) Լիմֆեդեմա
    է) Կանգայինսրտայինանբավարարություն
    ը) Ռևմատոլոգիական հիվանդություններ (վասկուլիտ, սկլերոդերմա, համակարգայինկարմիր գայլախտայլն)
    թ) Չարորակնորագոյացություններ
    ժ) Ռադիոթերապիա և իմունոսուպրեսիվ թերապիա

ի) Խոցեր և ամպուտացիաներ այլ պատճառներով բացիշաքարայինդիաբետից

9. Մահ

10. Այլ _____ (մանրամասներ)

Appendix 11 Tentative timeframe

(December-May)