What is the role of local inflammation in the development of diabetic foot ulcers? A systematic review

Inflammatory cytokines have been shown to contribute to diabetic pathological processes, such as neuropathy and nephropathy. Inflammatory markers play an important role in facilitating the diagnoses of diabetic foot infection. The aim of this review was to explore the published literature to determine the role of local inflammation in the development of diabetic foot ulcers. A systematic search of the literature was conducted using OVID Medline, Ovid EMBASE, EBSCO CINAHL Plus and Scopus in August 2020. A total of 326 records were screened, with six studies were eligible for inclusion. The evidence-based librarianship (EBL) checklist assessed the methodological quality of the studies included. The six studies were conducted between 2009 and 2020, and 67% (n=4) used a cross-sectional design. The mean sample size was 164 participants (standard deviation: ±11.8). Statistical significant differences were found among inflammatory mediators between controls and people with diabetes at risk of ulceration for hsCRP, TNF-α, IL-6, sE-selectin, IL-8, G-CSF, fibrinogen, sICAM-1, EGF and sVCAM-1. Statistically significant differences were found among people with diabetes with diabetic foot ulcers (DFUs) compared with patients without DFU for CRP, hsCRP, fibrinogen, TNF-α, IL-6, IP-10, MIF, MIP-1α, RANTES, HMGB1, VEGF, AOPP, MIP-1β, IFN-γ and EGF. The EBL score varied between 60%–85%. In total, 83% (n=5) of the studies scored ≥75%, reflecting validity. Altered levels of inflammatory mediators correlate with the risk of developing a diabetic foot ulcer. The implementation of a reliable measure that identifies diabetics at risk of developing diabetic foot ulcers through inflammatory mediators, may help decrease the incidence of this complication and its associated healthcare-related costs.

Ashley Lanys *is a Medical Student, The Royal College of Surgeons in Ireland (RCSI), University of Medicine and Health Sciences, Dublin;* ***Zena Moore*** *is a Professor;* ***Pinar Avsar*** *is a PhD Candidate;* ***Tom O'Connor*** *is a Professor;* ***Linda Nugent*** *is a PhD Candidate;* ***Pauline Wilson*** *is a Podiatrist & PhD Candidate;* ***Declan Patton*** *is a Professor; all at Skin, Wounds and Trauma Research Centre, School of Nursing and Midwifery, RCSI, University of Medicine and Health Sciences, Dublin*

Diabetes affects 422 million people worldwide and accounts for 1.6 million deaths each year (World Health Organization [WHO], 2021). The global prevalence is expected to rise to 600 million people by 2035 (Bakker et al, 2015). Diabetes has a wide array of macro- and microvascular complications, including cardiovascular disease, retinopathy, nephropathy and neuropathy (Cade, 2018). Diabetic foot ulcer (DFU) problems represent an onerous burden as they contribute to significant patient suffering and healthcare-related costs (Raghav et al, 2018). As a result of diabetic foot problems, a lower-limb amputation occurs every 30 seconds (Yazedanpanah et al, 2017). DFUs are responsible for 85% of amputations in this patient population (Zhang et al, 2017).

DFUs represent a serious complication that can arise from peripheral sensory neuropathy, foot deformities, minor foot trauma and peripheral arterial disease (Bakker et al, 2015). The underlying pathophysiological processes have neuropathic, vascular and immune system components (Wade and Dollahite, 2015). The accumulation of inflammatory cytokines leading to sustained inflammatory responses has been cited as one of the most important factors contributing to DFU development (Miao et al, 2020). This multifactorial aetiology generates challenges for treatment and, therefore,

highlights the importance of prioritising preventative measures (Iraj et al, 2013).

There are a number of emerging technologies that can help quantify the risk of developing DFU, which can be beneficial for prevention and management strategies. These include Doppler flowmetry, which assesses tissue viability; plantar pressure and pressure gradient systems to identify what specific sites are at risk for DFU; ultrasound identification to evaluate tissue mechanical properties; and infrared thermography for the early detection of inflammation (Lung et al, 2020). Infrared thermography examines the surface of the foot and identifies cold or hot spots, which specify where inflammation or circulatory processes may be occurring (Bharara et al, 2012).

One of the earliest signs of DFU is inflammation (Bharara et al, 2012). Inflammatory cytokines have been shown to contribute to diabetic pathological processes, such as neuropathy and nephropathy (Doupis et al, 2009, Navarro-Gonzalez et al, 2008). Interleukin 1 (IL-1), interleukin 6 (IL-6), interleukin-18 (IL-18), and tumor necrosis factor-alpha (TNF-α) are inflammatory cytokines that are known to be involved in the pathogenesis of diabetic nephropathy (Amsen et al, 2009). Enzyme-linked immunosorbent assays (ELISA) is one method that is commonly used to measure levels of inflammatory cytokines (Amsen et al, 2009). Inflammatory biomarkers are simple and inexpensive interventions that can assist in the early diagnosis of diabetic foot infections (Majeed et al, 2018).

A study examining levels of inflammatory biomarkers in people with diabetes with DFU and people with diabetes without DFU revealed that the presence of IL-6, C-reactive protein (CRP) and fibrinogen may play a role in the development and pathogenesis of DFU (Sallam, 2012). Another study found that patients with a DFU experience acute phase reactions through CRP and fibrinogen (Upchurch et al, 1997). Similarly, a study evaluating 10 subclinical inflammatory markers found high levels of CRP and IL-6 in people with diabetes with neuropathy (Herder et al, 2009). Therefore, there is support for exploring the use of inflammatory biomarkers as an indicator of subclinical inflammatory processes occurring within this patient demographic (Herder et al, 2009).

While there is an abundance of literature on the inflammatory processes behind diabetic wound healing, there is a paucity of literature on the role of inflammation in the development of DFU (Weigelt et al, 2009). Previous research has highlighted an interest in investigating whether activation of the immune system precedes the development of DFU, which could potentially have an anti-inflammatory therapeutic benefit (Weigelt et al, 2009). The purpose of this review is to systematically explore the role of local inflammation in the development of DFU. By identifying local inflammatory biomarkers behind the DFU pathophysiological processes, this review seeks to highlight a potential prognostic value that can be applied to improve therapeutic strategies in the care and management of people with diabetes.

Aim

The objective of this systematic review was to explore the published evidence exploring the role of local inflammation in the development of DFUs. The research question was as follows: "What is the role of local inflammation in the development of diabetic foot ulcers?"

Methods

Criteria for considering studies for this review

The systematic review included original research studies using a prospective design, and human studies written in English. Retrospective studies, conference papers, opinion papers, and qualitative methodology were excluded. No restrictions on the date of publication and study setting were applied.

Inclusion criteria

To be eligible for this review, studies had to include adult participants with type 1 or 2 diabetes, with a current DFU or being monitored for the development of a DFU. Studies monitoring inflammation through inflammatory mediators, or biomarkers were eligible for inclusion.

Exclusion criteria

Excluded studies included those that focused on the inflammation process behind the healing of a DFU, rather than on the development of a DFU.

Outcomes

The primary outcome of interest was the role of inflammatory mediators in the development of a DFU. The secondary outcome of interest was to examine the role of inflammatory mediators comparing people with diabetes with and without DFU.

Electronic searches

The following electronic databases were searched to identify relevant literature:

- Ovid MEDLINE (1946 to search date August 2020)
- Ovid MEDLINE (In-Process & Other Non-Indexed Citations) (latest issue)

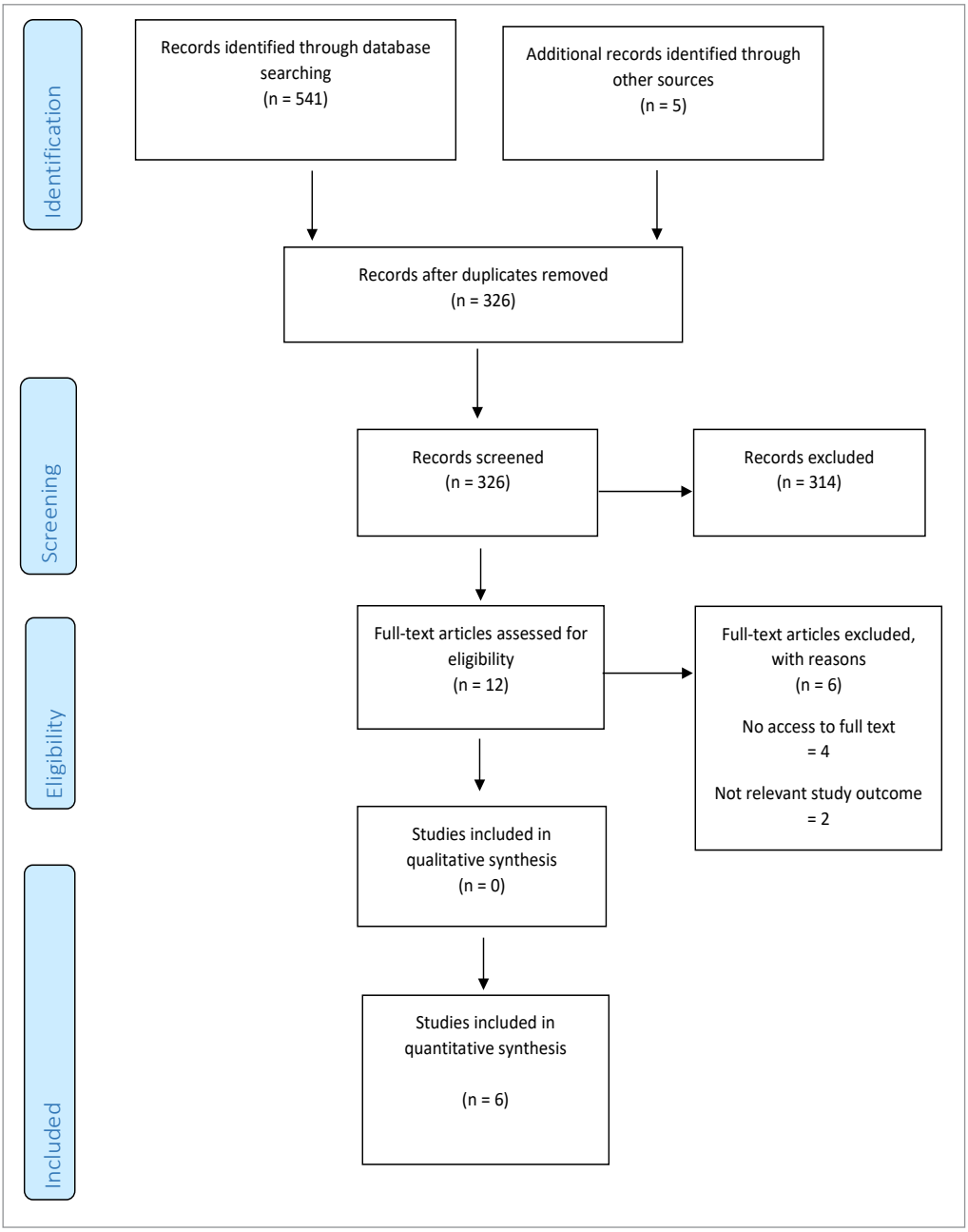

Figure 1. PRISMA flow diagram for study selection.

- Ovid EMBASE (1974 to search date August 2020);
- EBSCO CINAHL Plus (1937 to search date August 2020)
- Scopus.

To further identify the studies, the reference lists of all identified studies were scanned. Controlled vocabulary terms including medical subject headings (MeSH) for MEDLINE and Emtree for EMBASE were used. The search strategy included the following keywords:

#1 "diabetic foot"

#2 "foot ulcer"

#3 "Subclinical inflammation" OR "Inflammatory biomarker" OR "Inflammatory marker" OR "Inflammatory mediator" OR "Monocyte" OR "Neutrophil" OR "'Interleukin" OR "Adhesion molecule" OR "Adipokine" OR "Acute phase protein" OR "cytokine" OR "macrophage" OR "chemokine"

4: 1 # 2 # 3.

Study selection

Figure 1 outlines the flow of articles through the review. The article titles and abstracts that were identified by the search strategy were screened by two authors (AL, PA) independently, according to the studies' eligibility criteria. The screening of full text potentially relevant studies was completed by two reviewers (AL, PA) independently. Where discrepancies occurred

Table 1. Excluded studies.

Author	Study	Reason for exclusion
Miao et al, 2020	Decreased plasma maresin 1 concentration is associated with diabetic foot ulcer	Not relevant study outcome. Maresin 1 is a mediator of inflammatory resolution and thus focuses on anti-inflammatory mechanisms. This paper is interested in pro-inflammatory mediators and the inflammation process
Ozenc et al, 2012	Serum magnesium levels are significantly correlated with the severity of inflammation in patients with diabetic foot	Could not access full text
Umapathy et al, 2013	Is (TNF-α -308 g>a &-238 g>a) promoter polymorphism a major risk factor to be associated with neuropathic foot ulcer in an Asian Indian population?	Could not access full text
Umapathy et al, 2016	NRF-2 gene polymorphism is associated with an increased risk of diabetic foot ulcer among South Indian population	Could not access full text
Wang et al, 2018	The roles of NLRP3 inflammasome and ADAR1 in diabetic foot ulcer	Could not access full text
Dhamodharan et al, 2019	Circulatory levels of B-cell activating factor of the TNF family in patients with diabetic foot ulcer: Association with disease progression	No relevant study outcome. The study groups of analysis include diabetics with infected and non-infected foot ulcers. This focus on infection is outside the scope of inflammatory process leading to diabetic foot ulceration

between reviewers, a consensus was obtained through discussion.

Data extraction

Data from the included studies were extracted and inserted into a table using the following headings: study name, author, date of study, country, setting, sample size, design, participants, intervention, outcomes, results and level of evidence.

In this review, following the extraction of the main findings from the papers, meta-analysis statistical synthesis was considered inappropriate. Thus, first, the data were narratively summarised, giving an overview of the study setting, geographical location, study settings, sample sizes, instrument and results. This was followed by quality appraisal and a structured narrative synthesis of the six included studies, based on the outcome measures. The studies were quality appraised using the evidence-based librarianship (EBL) critical appraisal checklist (*Table 1*; Glynn, 2006). This quality appraisal tool assesses the validity, applicability, and appropriateness of a study, based on four main steps of the research process: population, data collection, study design, and results. According to this checklist, if the overall validity of the study (Yes/Total) is ≥75% or (No+Unclear)/Total) is ≤25%, then the study is valid (Glynn, 2006).

RESULTS

Overview of all included studies

Figure 1 outlines the flow of articles through the review. Following review of titles and abstracts from 546 citations, 534 were excluded. Then, following a review of the full papers of the remaining citations, six were rejected for the following reasons: not a relevant study outcome (Dhamodharan et al, 2019; Miao et al, 2020) and unable to access the full text (Ozenc et al, 2012, Umapathy et al, 2013; 2016; Wang et al, 2018) (*Table 1*). Finally, six articles were deemed to meet the inclusion criteria (an overview of the

Table 2. Study characteristics.

Author(s) & country	Design	Study setting	Sample size	Intervention
Weigelt et al, 2009 (Germany)	Cross-sectional study	Diabetes clinic	310	CRP and fibrinogen were measured in plasma samples with a high sensitivity latex-enhanced nephelometric assay on a BN II analyzer (Dade Behring, Marburg, Germany) using immunonephelometry, respectively. Serum levels of the cytokines interleukin-6 (IL-6), macrophage migration inhibitory factor (MIF), and regulated on activation, normal T cell expressed and secreted (RANTES) were determined using ELISAs (for IL-6 from Sanquin [Amsterdam, the Netherlands] and for MIF and RANTES from R&D Systems [Wies- baden, Germany]). Serum levels of IL-8, IL-alpha, and interferon-gamma-inducible protein — 10 were quantified using a bead-based multiplex assay on a Luminex 100 analyzer (Luminex, Austin, TX) 18, monocyte chemoattractant protein-1, macrophage inflammatory protein-1
Sallam and El-Sharawy, 2012 (Egypt)	Cross-sectional study	Hospital	51	IL-6 was determined by an ELISA; CRP was evaluated by immunoturbimetric assay and fibrinogen by immunoephelometric analysis
Zubair et al, 2012 (India)	Prospective Cohort Study	Hospital	324	Plasma levels IL-6, hsCRP, and TNF-α were measured by immunoenzymatic enzyme-linked immunosobrent assay method
Dinh et al, 2012 (USA)	Prospective Cohort Study	Foot Center and Clinical Research Center	144	Serum was analysed for the measurements of inflammatory cytokines, growth factors, and biochemical markers of endothelial function using a Luminex 200 apparatus (Luminex, Austin, TX) and Millipore multiplex immunoassay panels (Millipore, Chicago, IL)
Hafez et al, 2018 (Egypt)	Cross-sectional study	Endocrinology and Diabetes Unit	75	Enzyme-linked immunosorbent assays were used for measuring serum levels of TNF-α (Boster biological technology, USA), IL-6 (Boster biological technology, USA) and HMGB1 (bioassay technology laboratory, Shanghai, china) using ELISA reader
Ab El-Khalik et al, 2020 (Egypt)	Cross-sectional study	Endocrinology and Diabetes Unit	80	Serum samples were used for assay of advanced oxidation protein products (AOPPS), levels spectrophotometrically. Enzyme-linked immunosorbent assays were used for measuring plasma levels of TNF-α (Boster biological technology), and VEGF.

six studies is provided in Table 2 (Weigelt et al, 2009, Sallam and El-Sharawy, 2012, Zubair et al, 2012, Dinh et al, 2012, Hafez et al, 2018, Abd El-Khalik et al, 2020).

Study design

The studies were conducted between 2009 and 2020. Of the included studies, 67% (n=4) used a cross-sectional design (Weigelt et al, 2009, Sallam and El-Sharawy, 2012, Hafez et al, 2018, Abd El-Khalik et al, 2020) and 33% (n=2) used a prospective cohort study design (Dinh et al, 2012, Zubair et al, 2012).

Geographical location

The geographical location of the studies varied between Germany (17%; n=1) (Weigelt et al, 2009), Egypt (50%; n=3) (Sallam and El-Sharawy, 2012, Abd El-Khalik et al, 2020, Hafez et al, 2018), India (17%; n=1) (Zubair et al, 2012) and the US (17%; n=1) (Dinh et al, 2012).

Study settings

The study settings included a diabetes clinic, hospital, foot centre and clinical research centre, and endocrinology and diabetes unit (*Table 2*). The most common settings were a diabetes unit/clinic (n=3) and a hospital (n=2).

Sample size

The mean sample size was 164 participants (SD=±11.8), varying between 51 participants (Sallam and El-Sharawy, 2012) and 324 participants (Zubair et al, 2012).

Population

The population of interest was people with diabetes being monitored for the development of a DFU, or people with diabetes with a DFU. Three studies compared two groups which included people with diabetes with foot ulcers and people with diabetes without foot ulcers (Weigelt et al, 2009; Sallam and El-Sharawy, 2012; Zubair

et al, 2012). These groups were also analysed in addition to a third group of healthy controls in two studies (Hafez et al, 2018; Abd El-Khalik et al, 2020). One study included people with diabetes categorised as either high risk for developing a foot ulcer, low risk for developing a foot ulcer and healthy controls (Dinh et al, 2012). In the same study, a subsequent analysis compared people with diabetes who went on to develop a foot ulcer to people with diabetes without a foot ulcer (Dinh et al, 2012).

Results for the primary outcome

The primary outcome of this systematic review was an assessment of inflammatory mediators in the development of DFU. Only one study explored the role of inflammatory mediators in people with diabetes prior to developing a foot ulcer (Dinh et al, 2012). A total of 19 inflammatory biomarkers were analysed (Dinh et al, 2012). Levels of these biomarkers were assessed at different time points and as a result group classification and analysis varied. Initially, these biomarkers were measured in three groups: controls, people with diabetes who were at high risk of developing an ulcer and people with diabetes who were classified as low risk for ulcer development.

Statistically significant differences were found between controls and high-risk people with diabetes for high sensitivity c-reactive protein (hsCRP), TNF-α, IL-6, soluble E-selection (sE-selectin); between control and low-risk people with diabetes for IL-8; and between controls compared to low and high-risk people with diabetes for granulocyte-colony stimulating factor (G-CSF), fibrinogen and soluble intercellular adhesion molecule 1 (sICAM-1) (Dinh et al, 2012). Furthermore, statistically significant differences were found between low-risk subjects compared to controls and high-risk subjects for epidermal growth factor (EGF), and for low risk compared to high risk for soluble vascular cellular adhesion molecule-1 (sVCAM-1) (Dinh et al, 2012).

Results for the secondary outcome

The secondary outcome of interest sought to evaluate how inflammatory mediators differ between people with diabetes with and without DFU. Six studies explored the secondary outcome.

In addition to exploring the primary outcome, Dinh et al (2012) analysed people with diabetes who developed a DFU and compared them to those without a DFU. Dinh et al (2012) reported differences among these groups for EGF, transforming growth factor-alpha (TGFa), vascular endothelial growth factor (VEGF), G-CSF, hsCRP, TNFα, IL-6, IL-8, IL-2 receptor serum, macrophage inflammatory protein-1 alpha (MIP-1α), macrophage inflammatory protein-1beta (MIP-1β), monocyte chemoattractant protein-1 (MCP-1), interferon-gamma (IFN-γ), matrix metallopeptidase 9 (MMP-9), and total plasminogen activator inhibitor-1 (tPAI-1). Weigelt et al (2009) looked at CRP, fibrinogen, IL-6, macrophage migration inhibitory factor (MIF), interferon gamma-induced protein 10 (IP-10), MIP-1α, interleukin-18 (IL-18), IL-8, MCP-1, and regulated on activation, normal T-cell expressed and secreted (RANTES), and compared these markers in people with diabetes with ulcers to people with diabetes without ulcers. Sallam and El-Sharawy (2012), analysed IL-6, CRP, and fibrinogen plasma levels in people with diabetes without a foot ulcer compared to people with diabetes with a foot ulcer. Zubair et al (2012) investigated IL-6, hsCRP, and TNF-a in DFU patients compared to diabetic controls. Hafez et al (2018) looked at high mobility group box 1 (HMGB1), TNF-α, and IL-6 in healthy subjects, people with diabetes without DFU, and in people with diabetes with a DFU. Abd El-Khalik et al (2020) looked at TNFα, VEGF, and advanced oxidation protein products (AOPP) in healthy controls, diabetes without a DFU, and people with diabetes with a DFU.

There were 23 different biomarkers measured with the most common being TNFα, IL-6, and CRP. Statistically significant differences were found among people with diabetes with and without DFU for CRP (Sallam and El-Sharawy, 2012, Weigelt et al, 2009), hsCRP (Zubair et al, 2012), fibrinogen (Weigelt et al, 2009; Sallam and El-Sharawy, 2012) TNFα (Zubair et al, 2012; Hafez et al, 2018; Abd El-Khalik et al, 2020), IL-6 (Weigelt et al, 2009; Sallam and El-Sharawy, 2012; Dinh et al, 2012; Hafez et al, 2018; Abd El-Khalik et al, 2020), IP-10 (Weigelt et al, 2009), MIF (Weigelt et al, 2009), MIP-1α (Weigelt et al, 2009, Dinh et al, 2012), RANTES (Weigelt et al, 2009), HMGB1 (Hafez et al, 2018), VEGF (Abd El-Khalik et al, 2020), AOPP (Abd El-Khalik et al, 2020), EGF (Dinh et al, 2012), IFN-gγ (Dinh et al, 2012) and MIP-1β (Dinh et al, 2012).

Quality appraisal of included studies

The EBL Appraisal checklist was used to assess the methodological quality of the included studies in this systematic review, by focusing on the four main domains: population, data collection, study designs, and results (Glynn 2006). The mean validity score for all studies was 78% (SD: 8.6%). The minimum score was 60% (Sallam and El-Sharawy, 2012) and the highest score for overall validity was 85% (Weigelt et al, 2009). *Table 2* reveals that 83% of these studies

scored ≥75%, indicating that these studies were considered valid.

All studies contained methodological issues according to EBL's appraisal checklist. In the population domain, the main areas of concern that arose in almost all studies were that the inclusion and exclusion were not definitely outlined. Additional shortcomings included obtaining informed consent, and having a sample size large enough for sufficient estimates. In the data domain, the primary concern for all studies was whether or not data collectors were involved in the delivery of service to the target population. One study additionally did not describe their data methods clearly (Sallam and El-Sharawy, 2012). The study design domain had the highest validity scores, with shortcomings including missing statements of ethics approval and methodology stated in a way that would not allow replication. Finally, in the results domain, the main areas of concern were related to subset analysis being a minor, rather than major focus, including suggestions for further research and external validity.

DISCUSSION

The goal of this review was to explore the published literature to assess what role inflammatory mediators play in the pathogenesis of DFU. A total of six studies with a cumulative sample size of 984 participants were analysed to answer this question. Of the six studies, one study investigated the primary outcome, which was to evaluate the role of inflammatory mediators in the development of DFU (Dinh et al, 2012). In addition to the Dinh et al (2012) study, the remaining five studies explored the level of inflammatory biomarkers between people with diabetes with and without DFU (Weigelt et al, 2009; Sallam and El-Sharawy, 2012; Zubair et al, 2012; Hafez et al, 2018; Abd El-Khalik et al, 2020).

The primary outcome assessed inflammatory mediators that may play a role in the development of DFU. Of the 19 biomarkers assessed, nine inflammatory mediators differed significantly at baseline among controls, low-risk people with diabetes and high-risk people with diabetes (Dinh et al, 2012). At a second time point, Dinh et al (2012) compared people with diabetes who developed DFU and compared them to people with diabetes without DFU (Dinh et al, 2012). The most significant findings at this second-time point were a reduction in EGF, IFN-γ, MIP-1α, MIP-1β and IL-6 in those who developed DFU compared to people with diabetes without DFU (Dinh et al, 2012). This study revealed that changes in inflammatory levels were observed in participants' serum at baseline, which was 8 months before the development of a foot ulcer (Dinh et al, 2012). This indicates that the pathophysiological process underlying the development of a DFU can be revealed through levels of inflammatory mediators (Dinh et al, 2012).

This finding highlights that there could be a crucial window of opportunity for intervention to prevent DFU formation among high-risk patients. Since inflammatory mediators highlight marked changes in advance of a DFU, they could potentially be used as a screening diagnostic to guide intervention and management. Early detection of a DFU is important as it represents a burdensome complication of diabetes that can lead to significant pain and suffering (Raghav et al, 2018). A reliable measure that identifies people with diabetes at risk of a DFU, could decrease the incidence and prevalence of this complication and in turn, decrease healthcare related costs for its management. Overall, the implementation of a reliable screening diagnostic that measures inflammatory markers may decrease the prevalence of DFU.

Since it has already been shown that inflammatory mediators demonstrate a marked change months before a foot ulcer develops (Dinh et al, 2012), and that the measurement of inflammatory markers are simple and inexpensive (Majeed et al. 2018), follow-up studies should explore the use of inflammatory markers as a prognostic tool for the development of DFU. Research highlighting the reliability and validity of this intervention could have significant clinical and health care-related outcomes. With an attempt to decrease the incidence and prevalence of DFU, additional studies could determine whether knowledge of increased inflammatory markers in at-risk diabetic populations could be used as a preventative care intervention. This could help ascertain whether knowledge of increased inflammatory biomarkers translates into improved preventative practice and patient outcomes.

The secondary outcome sought to assess how inflammatory mediators differ among people with diabetes with DFU compared to people with diabetes without DFU. Out of 23 biomarkers, 15 were found to be significantly different between groups. CRP, hsCRP, fibrinogen, TNF-α, IL-6, IP-10, MIF, MIP-1α, HMBG1 and AOPP were found to be higher in people with diabetes with DFU compared to those without; whereas VEGF, RANTES, MIP-1b, IFN-γ, and EGF were found to be lower in people with diabetes with DFU, compared with people with diabetes without DFU.

The EBL appraisal checklist was used to evaluate the methodological quality of the included studies. Overall 83% of the studies were not clear in outlining their inclusion and exclusion criteria

(Sallam and El-Sharawy, 2012, Weigelt et al, 2009, Abd El-Khalik et al, 2020, Dinh et al, 2012, Zubair et al, 2012).

The eligibility criteria should be clearly established, and reported, as it influences the population and sample that will be selected. This is essential in order to adequately reflect the defined research question, and allow for study replicability (Glynn, 2006). For every study included, it was not clear if the data collectors were involved in the delivery of service to the target population. It is therefore unknown if those collecting the data were biased, which could have impacted the reliability of the results (Glynn 2006). For the study design domain, 50% of studies did not include a reference of ethics approval. For the results domain 83% of studies did not have a subset analysis as focusing the discussion of results on all subgroup was not particular. Despite these limitations, the EBL appraisal checklist has identified that 83% of these studies were valid overall.

The literature on the inflammatory process behind the healing of DFU is robust. However, there is paucity of research on the role of inflammation in the development and pathogenesis of DFU. This represents a significant gap in the literature, and identifies an important opportunity for further research and inquiry which could result in a decreased prevalence of DFUs.

Finally, a number of important limitations need to be considered. First, one of the main limitations of this review was a lack of literature exploring the use of inflammatory mediators in the pathophysiological process of DFU development. Due to a lack of data, the six studies that measured inflammatory mediators in the patient population of interest is not adequate to support the efficacy and integration of utilising inflammatory markers in clinical practice as a screening tool for the standard of care preventative measures. However, the results do show significant differences among different patient groups, which does provide rationale for further research.

Secondly, the limitation of this systematic review was the broad methodological heterogeneity of the included studies, which prevented the comparison between studies. A number of different inflammatory biomarkers and mediators were used across the six included studies, and, this heterogeneity meant that meta-analysis could not be completed for the primary and secondary outcomes of interest. However, five different databases were thoroughly searched to provide a comprehensive assessment of which inflammatory mediators may be utilised to predict the development of DFU.

CONCLUSION

This systematic review has identified that changes in inflammatory mediators correlate with people with diabetes who are at risk for developing DFU. This review provides future direction for research inquiry by highlighting a significant gap in the literature. The implementation of a reliable measure that identifies people with diabetes at risk of developing DFUs through inflammatory mediators, may decrease the incidence and prevalence of this complication and its associated healthcare-related costs. WAS

REFERENCES

Abd El-Khalik SR, Hafez YM, Elkholy RA (2020) The role of circulating soluble fms-like tyrosine kinase-1 in patients with diabetic foot ulcer: A possible mechanism of pathogenesis via a novel link between oxidative stress, inflammation and angiogenesis. *Microvasc Res* 130: 103987

Amsen D, de Visser KE, Town T (2009) Approaches to determine expression of inflammatory cytokines. *Methods Mol Biol* 511: 107–42

Bakker K, Apelqvist J, Lipsky BA et al (2015) The 2015 IWGDF guidance documents on prevention and management of foot problems in diabetes: development of an evidence-based global consensus. *Diabetes Metab Res Rev* 32 (Suppl 1): 2–6

Bharara M, Schoess J, Armstrong DG (2012) Coming events cast their shadows before: detecting inflammation in the acute diabetic foot and the foot in remission. *Diabetes Metab Res Rev* 28(Suppl 1): 15–20

Cade T (2008) Diabetes-related microvascular and macrovascular diseases in the physical therapy setting. Phys Ther 88(11): 1322–335

Dinh T, Tecilazich F, Kafanas A et al (2012) Mechanisms involved in the development and healing of diabetic foot ulceration. *Diabetes* 61(11): 2937–47

Dhamodharan U, Teena R, Vimal Kumar R et al (2019) Circulatory levels of B-cell activating factor of the TNF family in patients with diabetic foot ulcer: Association with disease progression. *Wound Repair Regen* 27(5): 442–9

Doupis J, Lyons T, Wu S et al (2009) Microvascular reactivity and inflammatory cytokines in painful and painless peripheral diabetic neuropathy. *J Clin Endocrinol Metab* 94(6): 2157–63

Glynn L (2006) A critical appraisal tool for library and information research. *Libr Hi Tech* 24(3): 387–99

Hafez YM, El-Deeb OS, Atef MM (2018) The emerging role of the epigenetic enzyme Sirtuin-1 and high mobility group Box 1 in patients with diabetic foot ulceration. *Diabetes Metab Syndr* 12(6): 1065–70

Herder C, Lankisch M, Ziegler D et al (2009) Subclinical Inflammation and Diabetic Polyneuropathy. *Diabetes Care* 32(4): 680–2

Iraj B, Khorvash F, Ebneshahidi A et al (2013) Prevention of diabetic foot ulcer. *Int J Prev Med* 4(3): 373–6

Lung C, Wu F, Liao F et al (2020) Emerging technologies for the prevention and management of diabetic foot ulcers. *J Tissue Viability* 29(2): 61–8

Majeed A, Mushtaq A, Iftikhar A et al (2018) Role of inflammatory markers in diagnosing diabetic foot infection: a meta-analysis. *Open Forum Infect Dis* 5(Suppl

1): S122–3

Miao T, Huang B, He N et al (2020) Decreased plasma maresin 1 concentration is associated with diabetic foot ulcer. *Mediators Inflamm* 2020: 4539035

Navarro-Gonzalez JF, Mora-Fernandez C (2008) The role of inflammatory cytokines in diabetic nephropathy. *J Am Soc Nephrol* 19(3): 433–42

Ozenc S, Yesilkaya S, Cakar M et al. (2012) Serum magnesium levels are significantly correlated with the severity of inflammation in patients with diabetic foot. *Diabetes* 61(Supplement 1): A99–A166

Raghav A, Khan ZK, Labala EK et al (2018) Financial burden of diabetic foot ulcers to world: a progressive topic to discuss always. *Ther Adv Endocrinol Metab* 9(1): 29–31

Sallam A, El-Sharawy A (2012) Role of interleukin-6 (IL-6) and indicators of inflammation in the pathogenesis of diabetic foot ulcers. *Aust J Basic & Appl Sci* 6(6): 430–5

Umapathy D, Balashanmugam P, Dornadula S et al (2016) NRF-2 gene polymorphism is associated with an increased risk of diabetic foot ulcer among south indian population. *Diabetes* 65(Supplement 1): A360–431

Umapathy D, Vivekanandhan A, Krishnmoorthy E, et al. (2013). Is TNF-a -308 g>a &-238 g>a promoter polymorphism a major risk factor to be associated with neuropathic foot ulcer in an Asian Indian population? *Diabetes* 62(Supplement 1): A685–A718

Upchurch GR, Keagy BA, Johnson G (1997) An acute phase reaction in diabetic patients with foot ulcers. *Cardiovasc Surg* 5(1): 32–6

Wade A, Dollahite HA (2015) Pathogenesis and management of diabetic foot ulcers. *JAAPA* 28(5): 28–34

Wang G, Zhao L, Yang W et al (2018) The roles of NLRP3 inflammasome and ADAR1 in diabetic foot ulcer. *Diabetes* 67(Supplement 1): 642-P

Weigelt C, Rose B, Poschen U et al (2009) Immune mediators in patients with acute diabetic foot syndrome. *Diabetes Care* 32(8): 1491–6

World Health Organization (2021) Diabetes. https://bit.ly/2OZY9OK (accessed 16.03.2021)

Yazedanpanah L, Nasiri M, Adarvishi S (2017) Literature review on t*he management of diabetic foot ulcer.* World J Diabetes 6(1): 37–53

Zhang P, Lu J, Jing Y et al. (2017) Global epidemiology of diabetic foot ulcercation: a systematic review and meta-analysis. Ann Med 49(2): 106–16

Zubair M, Malik A, Ahmad J (2012) Plasma adiponectin, IL-6, hsCRP and TNF-a levels in subject with diabetic foot and its correlation with clinical variables in a North Indian tertiary care hospital. Indian J Endocrinol Metab 16(5): 769–76