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Review Article

Genetic Modifiers in Sickle Cell Disease Leg Ulcers: Unveiling the Pathways associated with the development and, or progression of Leg Ulcers - A Scoping Review Protocol.

***Livingstone Gayus Dogara[1], Sani Awwalu[2], Doyinsade Awodele[3].**

[1]Department of Haematology and Blood Transfusion, Faculty of Basic Clinical Sciences, Kaduna State University College of Medicine and Barau Dikko Teaching Hospital (BDTH) – Kaduna State University, Kaduna, Nigeria. [2]Department of Haematology and Blood Transfusion, Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Nigeria, [3]Global Medicine (GMED) Scholar, University of Illinois at Chicago, USA. On behalf of the International Hemoglobinopathy Research Network (INHERENT).

Abstract

This scoping review aims to assess the literature on genetic modifiers of leg ulcers in sickle cell disease, evaluating available evidence, methodologies, and research gaps. A major morbidity in sickle cell disease is the development of leg ulcers. This clinical syndrome of SCD leg ulcers (SLU) has continued to be an enigma due to its multifactorial evolution, dearth of promising guidelines on treatment, and generally unsatisfactory response to treatment. Underlying genetic susceptibilities for SLU may impact counselling, prognostication, risk of development, severity as well as response to interventions. Hence the need for this scoping review. This scoping review will collate and assess studies in English on genetic markers of SLU among all SCD age groups, genders, races, and regions. Genetic or molecular markers to be assessed among patients with sickle cell leg ulcers included, genetic markers of Inflammation, vasculopathy, tissue damage, oxidative stress, coagulopathy as well as genetic predispositions that have been studied in relation to SLUs across all countries. This includes most common biomarkers that promote development of SLU, the single nucleotide polymorphic markers (SNPs) that work through the MAPK and SMAD signaling pathway. A PubMed search of all fields for literature published in English using the strategy (sickle cell) AND (leg ulcer), and (sickle cell) AND (leg ulcer genetics) from 1998 to 2023 (last 25 years) will be undertaken. This will be modified, according to the inclusion criteria, as appropriate across other databases. The other databases will include Google Scholar, web of Knowledge, Scopus, New Zealand Science, Silver chair, Taylor and Francis+NEJM, and journals.lww.com.

Keywords: *Genetic; Genetic Modifiers; Markers; Genetic Biomarkers; Leg Ulcer; Scoping Review; Sickle Cell; Sickle Cell Disease.*

***Correspondence**: Livingstone Gayus Dogara, Department of Haematology and Blood Transfusion, Faculty of Basic Clinical Sciences, Kaduna State University College of Medicine and Barau Dikko Teaching Hospital (BDTH) – Kaduna State University, Kaduna, Nigeria.
Email: livingstone.dogara@kasu.edu.ng

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Introduction:

Sickle Cell Disease (SCD) is the most widely distributed and common single gene disorder which is the prototype disorder for study in chronic inflammatory conditions.[1] Its hallmark presentation is with painful bone crises. Despite concerted research efforts, it is still a disorder that has not been given the considered appropriate attention despite the World Health Organization's (WHO) pronouncement of it as a disease of public health importance in the world not only in Sub-Saharan Africa (SSA) where the major burden and severe phenotypes exist. The global disease burden report reported over half a million babies are born every year with SCD and 75% of them in Sub-Saharan Africa and over five million all-age disease prevalence.[2,3]Nigeria and the Demographic Republic of Congo (CDR) are the countries with the highest burden of the severest phenotypes: the Bantu and Benin haplotypes.[4,5] Early diagnosis and mapping of the genotype-phenotype profiles of the disease is an important process in developing a comprehensive standard of care that mitigates most of the adult-onset complications.[4,5] This approach is noted to contribute to longevity of persons living with SCD.[4,6,7]

It is estimated that of the 150,000 babies delivered yearly in Africa with SCD,[8,9] about 50% of them may not see their 10th birthday.[4,6,7] Those who survive up to adulthood develop a number of complications which are worsened by late diagnosis, occasioned by delayed presentation. This is further compounded by poor commitment to care because of lack of government support to the health needs of the individuals.[5,10] This could be because of competing needs or that of lack of established and robust health and social insurance schemes.[11]

A complex interplay of genetics and environmental factors lies at the heart of the multi-systemic course of SCD leading to challenges in defining the modifiers and biomarkers of the syndromes associated with SCD. One of such debilitating syndromes is chronic leg ulcers. Leg ulcers in SCD are the commonest cutaneous disorders and noted hallmarks of phenotypic severity of SCD.

Thus, SCD may be viewed as an avalanche of cascading genetic interactions; the primary genetic change resulting in SCD and other genetic variabilities leading to the evolution of additional presentations and complications. To develop a sustained care model for SLU, researchers need to know which genetic factors contribute most to these processes.

There have been great advances in the field of molecular genetics over the years and efforts have been on to study how genetics impact on SCD in general. It is important to assess the extent to which genetics affect SLU in general. As an example, the odds of having SLU have been shown to be higher among blacks compared to whites suggesting an underlying genetic risk factor.[12] Additionally, by utilizing whole exome sequencing among discovery and validation cohorts, the role of the FLG2 gene variants in SLU has been elucidated.[13] In addition to the roles of SNPs, larger mutations such as the co-existence of alpha gene deletions have been suggested to be protective of SLU.[14] Others yet still variations in incidence of SLU have been described depending on the number of alpha genes in SCD patients. Interestingly, a different group of genetic markers, circulating micro RNAs have been suggested to have either predisposing (miR-199a-5p, miR-144) or protective (miR-126) roles in SLUs.[15]

The predominant male preponderance of leg ulcers in SCD maybe attributed to physical activities.[4,16,17] These suggestions of physical activities do not preclude the possibility of genetic differences since not all males with SCD develop SLUs. The genetic modifiers are diverse and function at different stages in the evolution of this condition. While some help to silence the BCL11 gene, others enhance the elaboration of HbF levels or, serve as homeostatic factors in the system by providing buffering capacity to the red cells.[16,18] Additionally, some modifiers work to support the stability of blood vessels and interactions with the cellular membrane.[16,18]

It is of importance to categorize these genetic modifiers into those that either drive or protect local wound healing and those that look at the general systemic factors that may support or impair development of leg ulcers (generally sickle cell disease therapies).

This review is an attempt to look at the genetic modifiers within the context of the accepted definition and discuss of biomarkers; as "characteristics that are objectively measured and evaluated as indicators of normal biologic processes, pathological states, or pharmacologic responses to a therapeutic intervention."[19–21] Suffice to say, for leg ulcers in SCD, the discussion should and will reveal the genetic biomarkers that may appear either nonspecific to only SCD leg ulcers but may have overlapping roles in SCD clinical care and research in general.

There is also renewed interest in genotypic expressivity and penetrance in diverse medical conditions and SLU is not an exception. This lack of a primary mechanism which could serve as a prophylactic or therapeutic target in SCD leg ulcers is still an ongoing challenge. The two major phenotypic presentation of SCD, hemolytic and vasculopathy presentations, cannot on their own explain some of the more specific and severe presentation of some of the complications in SCD including the leg ulcers, therefore, the possibility of interactions and variability of all the factors may be a more acceptable explanation.[20,22] It is instructive to note variability in the disease processes, progress, and manifestation of complications in SCD which is supportive by the definition of the genotypes/phenotypes.

In a preliminary search of PubMed, Cochrane, JBI evidence synthesis and Medline no similar reviews were detected. However, an ongoing systematic review and meta-analysis protocol registered on PROSPERO on 03/11/2020 with CRD42020213310 was identified.[23] The authors plan to conduct a systematic review and meta-analyses on many aspects of SLU however they narrowed their objective in genetics to Single Nucleotide Polymorphisms (SNPs). Our protocol differs from this in the following aspects: we will be utilizing a scoping review as a first step based on the suggested decision tree for selecting scoping review by Pollock *et al*,[24] we are interested in all types of mutations (with SNPs being one type of mutation), all study designs and all types of literature including grey literature.

This scoping review's main objectives are to assess the availability, types, methodologies, gaps and quality of literature on all genetic mutations as markers of leg ulcers in sickle cell disease with a view to understanding the main trends in this field and determining the adequacy or otherwise of these literature in various forms of evidence syntheses.

Review questions

What is the nature of research on genetic profiles of leg ulcers in sickle cell disease? What is the research terrain on genetic modifiers that have been documented to either promote or prevent the development of SLUs? Are there genetic markers that improve response to therapeutic approaches in SLUs?

Keywords: Genetic, genetic modifiers, markers, genetic biomarkers, Leg ulcer, Scoping Review, Sickle cell, Sickle cell disease

Eligibility criteria

Participants: Studies on patients with sickle cell disease with leg ulcers will be collated.

Concept

Any genetic or molecular marker assessed among patients with sickle cell leg ulcers. These may include genetic markers of Inflammation, vasculopathy, tissue damage, oxidative stress, coagulopathy as well as genetic predispositions that have been studied in relation to SLUs. This broad categorization will include

majorly the genome wide association (GWAS) studies, as well as those that assess interleukins through the molecular laboratory protocol.

Context
Patients with SCD leg ulcers of all ages and across all countries.

Types of Sources
Literature utilizing any type of study design will be collated. These study designs will include observational, quasi-experimental and experimental. Systematic reviews, guidelines and expert opinion articles will be included. Both published and grey literature will be collated.

Methods

This scoping review will be guided by the JBI scoping review methodology,[25] following the PRISMA guidelines and registration information [24,26]A PubMed search of all fields for literature published in English using the strategy (sickle cell) AND (leg ulcer), and (sickle cell) AND (leg ulcer genetics) from 1998 to 2023 (last 25 years) will be undertaken. This will be modified, according to the inclusion criteria, as appropriate across other databases. The other databases will include Google Scholar, web of Knowledge, Scopus, New Zealand Science, Silverchair, Taylor and Francis+NEJM, and journals.lww.com

Search strategy
We will conduct a preliminary search to locate articles fulfilling the PCC (Population (or participants)/Concept/Context) framework of this scoping review. A full search strategy would be developed from these initial searches. This will be used to interrogate the following databases: PubMed, Google Scholar, Web of Knowledge, Scopus, New Zealand Science, Silverchair, Taylor and Francis+NEJM and Journals.lww.com. An iterative approach will be used to further search the reference list of all identified literature for further relevant studies. Our interest is in all types of mutations (with SNPs being one type of mutation), all study designs and all types of literature including grey literature. The protocol for this systematic review is registered on OSF accessed at https://osf.io/ux5wq/

Study/Source of Evidence selection
This scoping review will utilize the Preferred Reporting Items for Systematic Reviews and Meta-analyses extension for scoping review (PRISMA-ScR) flow diagram.[24,27] All literature obtained from the search will be imported into Zotero Version 7.0.6 (September 16, 2024)**.**[28,29]All duplicates will be removed and independent level one screening of titles and abstracts will be done by two reviewers. Thereafter, selected full text articles will be subjected to level two screening by two independent reviewers. Where there are differences between reviewers, a third reviewer will serve as a tiebreaker after review of the articles that passed the screening phase were imported into the created Zotero reference manager electronic library for retrieval of any missed additional studies.

Data Extraction and Charting
Two authors will conduct data charting, and a third author will review and in the event of no agreement, the third author serves as the tie breaker, based on the inclusion criteria for this scoping review. The data charting form that will undergo piloting is presented as Appendix I. This is modified from the JBI with items specific to the PCC of this scoping review added.[25,30] This may however be updated or modified during the review stage.

Data Analysis and Presentation
Microsoft Excel Microsoft® Excel® 2019 MSO (Version 2404 Build 17531.20210) and R on Julius.aifor data extraction, charting, from all included articles for quality; quality assessment, evidence synthesis, and for

descriptive formats as flow charts, tables, and graphs. All the included studies will be based on investigations or critical review of the role of genetic modifiers in the development and/or progression of leg ulcers in patients with SCD. Extracted information will include the following: i. study details (title, authors, year of publication); ii. study population, participant demographics; iii. phenotype studied; iv. gene and genetic variant studied; v. study design; vi. outcome studied, such as effect size (beta-values, odds ratio (OR), hazard ratio (HR)), frequencies or means in different groups; vii. statistics (method, multiple correction, adjusted covariates, p-value). If multiple models of inheritance are investigated, data for all models will be extracted.

Quality Assessment

Quality of the publications will follow a grade point system score of accessing journal and publication quality that categorizes quality as either; low, moderate, and high quality,[31,32] and with together with the Q-Genie assessment tool which uses a grade point system scores; ≤35 indicate poor-quality studies, >35 and ≤45 indicate studies of moderate quality, and >45 indicate good-quality studies. Case-control and cohort studies however will be rated from 0 to 9 using outcome of interest. A score of 0–2 is judged as poor quality, 3–5 as fair quality, and 6–9 as good quality.[33,34]

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