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Evaluation of haemoglobin in blister fluid as an indicator of paediatric burn wound depth

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ABSTRACT

The early and accurate assessment of burns is essential to inform patient treatment regimens; however, this first critical step in clinical practice remains a challenge for specialist burns clinicians worldwide. In this regard, protein biomarkers are a potential adjunct diagnostic tool to assist experienced clinical judgement. Free circulating haemoglobin has previously shown some promise as an indicator of burn depth in a murine animal model. Using blister fluid collected from paediatric burn patients, haemoglobin abundance was measured using semi-quantitative Western blot and immunoassays. Although a trend was observed in which haemoglobin abundance increased with burn wound severity, several patient samples deviated significantly from this trend. Further, it was found that haemoglobin concentration decreased significantly when whole cells, cell debris and fibrinous matrix was removed from the blister fluid by centrifugation; although the relationship to depth was still present. Statistical analyses showed that haemoglobin abundance in the fluid was more strongly related to the time between injury and sample collection and the time taken for spontaneous re-epithelialisation. We hypothesise that prolonged exposure to the blister fluid microenvironment may result in an increased haemoglobin abundance due to erythrocyte lysis, and delayed wound healing.

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1. Introduction

The depth or severity of a burn is used by clinicians as the predominant variable to predict the time for spontaneous re-epithelialisation and the associated scarring outcomes [1]. Many methods and technologies have been developed to assist in measuring burn wound depth and healing potential; however, most of these are applied primarily in research as they have substantial clinical limitations, such as the size and cost of instruments or a requirement for specialist training [2]. Thus the gold standard of clinical care in paediatric burns is visual assessment by a clinician, which is both subjective and heavily dependent on the clinician's training and experience. Therefore there remains the need for development of a more robust assessment tool.

Protein biomarkers have been investigated as an indicator of the presence or progression of many common conditions, such as osteoarthritis, autoimmune diseases and several cancers [3–8]. To date few quantitative biological indicators or markers have been investigated for skin related conditions or specifically for burn wounds. Burn patient serum has been investigated for biomarkers predicting survival in severely burnt patients [9]; but as the incidence of burn mortality in Australia is relatively low, the majority of paediatric patients presenting to Australian burn centres do not face these survival concerns [10,11]. Studies focussing on biomarkers that could assist in predicting cutaneous wound healing trajectories have predominantly been conducted with the aim of assessing chronic non-healing wounds rather than acute wounds [12–15]. Although burn wound exudate has been used as a healing wound comparator in some chronic wound focussed studies [16,17], it is unclear whether a similar approach could be applied when assessing acute burn wounds only.

Previously, the free circulating haemoglobin found in the plasma from a rat burn model has shown some promise as a biomarker of burn wound severity [18]; although this has not been further investigated in human patients. Moreover, the use of blood as a diagnostic sample is undesirable, particularly in the paediatric outpatient setting. In contrast to blood, blister fluid is readily available with minimal disruption to the patients or their medical treatment. As blister fluid is a plasma filtrate [19] proximal to the burn injury, there is potential for alterations in protein abundance which are detectable in blood to also be detectable in blister fluid. Blister fluid has previously been evaluated for its potential in biomarker discovery and measurement [20] and its utility in investigating the burn wound microenvironment [21], although it remains unclear whether changes in the local wound environment are detectable in this sample type or whether they would be masked by larger, systemic alterations. The ability to detect wound site-specific alterations in protein abundance may affect the ability of blister fluid to perform as a sample type for diagnostic or prognostic tests.

This study therefore aimed to evaluate the use of haemoglobin as an indicator of burn wound severity in a population of paediatric burn patients using wound exudate. Analysis of a subset of samples from patients with multiple burn sites was also conducted to determine whether site specific alterations in haemoglobin abundance could be detected.

2. Methods

2.1. Ethics statement

Ethical approval for this study was obtained from the Royal Children's Hospital (RCH) Human Research Ethics Committee (No. HREC/11/QRCH/189) and the Queensland University of Technology Human Research Ethics Committee (QUT HREC Approval No. 1200000038). Clinical and demographic data from patients enrolled in the study were collected at the time of consent and at subsequent clinical visits.

2.2. Sample collection and handling

Samples were collected through the Stuart Pegg Paediatric Burn Centre and the Department of Emergency Medicine at the RCH. During routine blister de-roofing procedures, fluid was acquired by either puncturing and aspirating the blister with a needle and syringe or puncturing the blister with scissors and collecting the fluid in a 200 μL ringcaps® capillary pipette (Hirschmann Laborgerate, Eberstadt, Germany). To investigate free haemoglobin compared to that contained within erythrocytes, 28 samples collected by capillary pipette were centrifuged at 855× RCF immediately following collection to remove cells and debris. Prior to and immediately following centrifugation, representative samples were viewed using a Nikon Eclipse Ti inverted microscope or a Nikon Eclipse microscope, at ×40 magnification and an aliquot of fluid was examined using a Neubauer chamber to perform erythrocyte counts. The pelleted cellular debris was stained with Giemsa and cell morphology was compared with a similarly stained whole blood sample. All samples were stored in aliquots at −80 °C. The total protein concentration of each sample was determined using the bicinchoninic acid (BCA) assay (Pierce, Rockford, USA), as per the manufacturer's instructions.

2.3. SDS PAGE and Western blot

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) gels were cast using the Bio-Rad mini Protean system (Bio-Rad, Hercules, USA). The resolving gel contained 375 mM tris(hydroxymethyl)aminomethane–hydrochloric acid (Tris–HCl) pH 8.8, 10% acrylamide/bisacrylamide (50:1) and 0.1% SDS in a total of 4.5 mL per gel. The stacking gel contained 375 mM Tris–HCl pH 6.8, 4% acrylamide/bisacrylamide (50:1) and 0.1% SDS in a total of 2 mL per gel. Polymerisation was catalysed by addition of tetramethylethylenediamine (TEMED) and ammonium persulphate (APS). Samples (10 μg) and lysed human erythrocytes (1 μg; positive control) were prepared in NuPAGE lithium dodecyl sulphate sample buffer containing 100 mM dithiothreitol, incubated for 10 min at 70 °C and subject to electrophoresis at 180 V for 50 min in Tris–glycine SDS running buffer (25 mM Tris, 190 mM glycine, 0.1% SDS). Precision Plus protein

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