Plasma Free Hemoglobin as A Diagnostic Tool for Assessment of Burn Depth

Original Article

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ABSTRACT

Background: A special interest has been drawn lately trying to fill some gaps in understanding the pathophysiology and the most suitable multidisciplinary modalities for burn care and management.

Objective: To detect correlation between plasma free hemoglobin (PFHb) levels and depth of burn to act as a prognostic indicator for burn treatment.

Patients and Methods: This observational study was conducted on 54 patients, presenting to Kasr EL Eini Cairo University Hospital seeking treatment for burn injuries, in the period between July 2018 and 2020.

Results: All patients showed moderate hemolysis and none showed severe hemolysis. There was statistically significant difference between deep and superficial burns as regard PFHb on admission, at 6 and 12h, and no statistically significant difference as regards PFHb at 48h. The correlation between total body surface area percentage and levels of PFHb on admission, at 6, at 12, and 48h of admission is a moderately positive correlation.

Conclusion: PFHb has the potential to be a useful diagnostic tool. This is the first laboratory test to show a link between depth of burn and total body surface area. It is suggested that the level of PFHb after burn injury is related to the depth and size of burn. So it is a valuable diagnostic tool in burn assessment.

Key Words: Burn Depth, Intravascular hemolysis, Plasma Free Haemoglobin.

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INTRODUCTION

A special interest has been drawn lately trying to fill some gaps in understanding the pathophysiology of burns and the most suitable multidisciplinary modalities for burn care and management[1].

Burn injuries are classified according to the depth of the injury to the skin and underlying structures. Previously, burns were classified as first, second, or third degree[2]. This classification has been replaced by a new system that classifies burns as either superficial, partial-thickness or full-thickness burn injury. The current gold standard method for estimation of burn size and depth is a subjective clinical assessment which even with the most experienced physicians still is not very accurate and at best is only 70% accurate. There are currently no biochemical or hematologic assays that can predict prognosis of burns according to burn depth nor detect the difference between deep dermal and full-thickness burn injuries[3].

Burns cause damage to the red blood cells which results in intravascular hemolysis and hemoglobin is usually released in plasma and can be tested for by measuring the hemoglobin dissolved in the plasma hence known as plasma free hemoglobin (PFHb)[4,5].

PFHb levels analysis showed promising results in animal models in burns as well as in other conditions in humans such as in sepsis, ECMO, medical devices surgery.

PFHb levels greater than 50mg/dl indicate severe haemolysis, between 5 and 50mg/dl indicate moderate hemolysis and normal/acceptable PFHb values are less than 5mg/dl). According to current findings, intravascular haemolysis is most likely mediated by systemic complement activation[4,5].

Currently, the gold standard method for estimating the depth and size of a burn is subjective clinical assessment

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that requires clinical knowledge and expertise. An early predictor assay that is inexpensive and widely available, that can detect burn depth and indicate whether this burn will heal within three weeks or not can be of great aid for burn assessment and hence act as a prognostic indicator for burns[6].

This work aimed to detect a correlation between PFHb levels and depth of burn to act as a prognostic indicator for burn treatment.

PATIENTS AND METHODS:

This observational study was conducted on 54 patients, presenting to Kasr EL Eini Cairo University Hospital seeking treatment for burn injuries, in the period between July 2018 and 2020. The sample population of subjects who suffered burns, was observed for analysis of intravascular hemolysis measured by PFHb levels.

Inclusion criteria

Patients with superficial and deep dermal burns and patients with less than 40% burns.

Exclusion criteria

Patients with extensive burns, electrical burns, chemical burns, comorbidities such as coagulation and bleeding disorders, uncontrolled diabetes, splenomegaly, liver diseases (hepatitis affects hemolysis), jaundice, leukemia, lymphoma, and autoimmune hemolytic anemia.

Methods

After primary survey and resuscitation of patients with fluids and stabilizing their condition, data from subjects fulfilling the inclusion and exclusion criteria was recorded such as age, sex, time of burn, type of burn, and percentage of total surface area. Four blood specimens were taken on admission, at 6,12, and 48h after admission. A 2ml of blood specimen volumes were obtained in a lavender top collection container with EDTA. Measurement of hemolysis was done by the spectrophotometric Harboe method using Abbott 4100 device where hemoglobin was quantified by measuring absorbance at three points (415,380, and 450nm). "Allen correction" was incorporated into a formula to convert absorbance measurements directly into Hb concentration:

$$\mathrm{Hb(g/l)} = (167.2 \times A_{415} - 83.6 \times A_{380} - 83.6 \times A_{450}) \times 1/1000 \times 1/\text{dilution in } dH_2O.$$

Samples were diluted in distilled water and incubated at room temperature for 30min to lyse red blood cells.

Follow-up

Follow-up of patients was for 2–3 weeks to monitor them for re-evaluation of clinical assessment of burn depth, those subjects who required ICU admission and management decisions for those subjects, whether nonsurgical or surgical as seen in Figures [1–4] below.

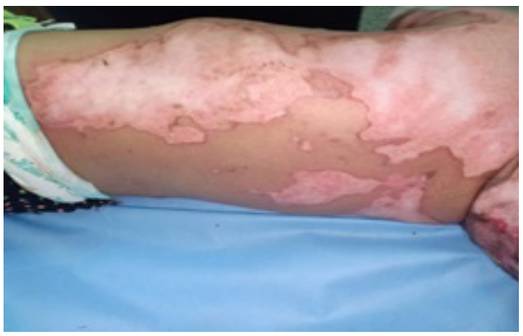

Figure 1: Patient 1 with deep dermal burn PFH 15.66, 16.75, 13.3 and 9.95mg/dL at admission, at 6hrs, at 12hrs and 48hrs after admission.

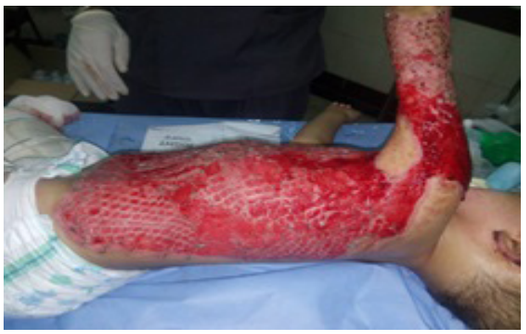

Figure 2: Patient 1 with deep dermal burn PFH 15.66, 16.75, 13.3 and 9.95mg/dL at admission, at 6hrs, at 12hrs and 48hrs after admission.

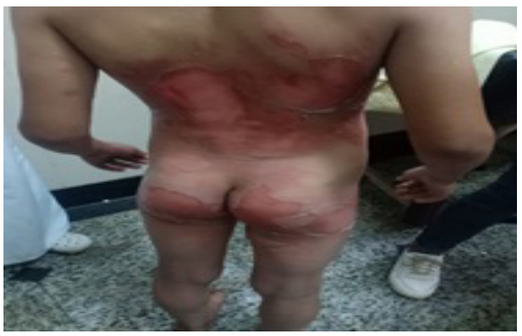

Figure 3: Patient 2 with superficial dermal burn, PFH 12.21, 13, 12.44 and 12.74mg/dL at admission, at 6hrs, at 12hrs and 48hrs after admission.

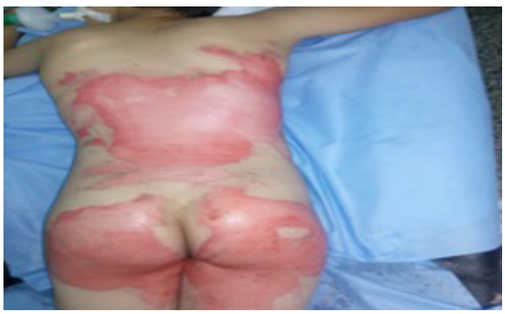

Figure 4: Patient 2 with superficial dermal burn, PFH 12.21, 13, 12.44 and 12.74mg/dL at admission, at 6hrs, at 12hrs and 48hrs after admission.

Statistical analysis

Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, New York, USA). Data was summarized using mean, SD, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (%) for categorical data. Comparisons between quantitative variables were done using the nonparametric Mann–Whitney tests. Correlations between quantitative variables were done using the Spearman correlation coefficient. Receiver operating characteristic curve was constructed with area under the curve analysis performed to detect best cut-off value of PFHb for the detection of deep burn and the need for ICU admission. *P* values less than 0.05 were considered as statistically significant.

RESULTS:

PFHb values up to 5mg/dl were acceptable, values between 5 and 50mg/dl indicated moderate intravascular hemolysis and values above 50mg/dl signify that haemolysis was severe. Subjects all showed moderate hemolysis and none showed severe hemolysis (Table 1).

Table 1: Severity of hemolysis:

Time Point	Category	Count	% of Total
Admission	**Normal**	**1**	**1.9%**
	Moderate hemolysis	53	98.1%
	Severe hemolysis	0	0.0%
6 Hours	**Normal**	**0**	**0.0%**
	Moderate hemolysis	54	100.0%
	Severe hemolysis	0	0.0%
12 Hours	**Normal**	**2**	**3.7%**
	Moderate hemolysis	52	96.3%
	Severe hemolysis	0	0.0%
48 Hours	**Normal**	**5**	**9.3%**
	Moderate hemolysis	49	90.7%
	Severe hemolysis	0	0.0%

Table (2) showed that there was statistically significant difference between deep and superficial burns as regard PFHb on admission, at 6 and 12h and no statistically significant difference as regard PFHb at 48h.

Table 2: Correlation between burn depth and plasma free hemoglobin:

	Depth										
	Deep (*n*= 33)					Superficial(*n*= 21)					*P* value
	Mean	SD	Median	Minimum	Maximum	Mean	SD	Median	Minimum	Maximum	
Plasma free haemoglobin on admission	15.58	6.59	13.48	8.34	38.70	12.55	2.29	12.21	7.48	18.38	0.032
Plasma free haemoglobin at 6 hours	16.22	6.63	14.31	8.20	39.72	12.92	1.80	12.72	8.33	16.26	0.012
Plasma free haemoglobin at 12 hours	14.98	6.56	12.97	9.20	38.21	11.64	2.75	11.78	1.83	15.76	0.045
Plasma free haemoglobin at 48 hours	13.07	6.04	11.48	6.53	36.00	10.69	2.15	10.90	7.00	15.71	0.081

Table (3) showed that PFHb values peaked at 6h with a mean of 14.93mg/dl, a median 13.29mg/dl, a minimum of 8.2mg/dl, and a maximum 39.72mg/dl. Lowest values were at 48h after admission, with a mean of 12.14mg/dl, a median 11.04mg/dl, a minimum of 6.53, and a maximum of 36mg/dl.

Table 3: Values of plasma free hemoglobin at different times:

	Mean	SD	Median	Minimum	Maximum
Plasma free hemoglobin on admission	14.40	5.51	12.70	7.48	38.70
Plasma free hemoglobin at 6 hours	14.93	5.51	13.29	8.20	39.72
Plasma free hemoglobin at 12 hours	13.68	5.62	12.40	1.83	38.21
Plasma free hemoglobin at 48 hours	12.14	5.01	11.04	6.53	36.00

DISCUSSION

Burns are a type of injury to skin and underlying structures caused by heat, chemicals, electricity, radioactivity, and friction[7].

Burns are a major public health issue accounting to 180,000 deaths yearly as well as a leading cause of morbidity, disability and disfigurement. They have a huge impact on the health of patients and quality of life, through its physical effect as well as its negative psychological effect it has on patients.

Burns have always been and still remain a challenging issue to manage, not only for its local effects but also for its systemic affection of most of the body systems. Hence a thorough knowledge and detailed understanding of all aspects of burns is needed by plastic surgeons in order to provide the best outcome for their patients and avoid or at least minimize the undesirable adverse effects that might range from bad scarring to endangering the patient's own life.

Burn depth is reflected on and defined by healing time and scar development. Scarring is unlikely if a partial-thickness burn heals within 2 weeks after a burn. Scarring is more likely between 2 and 3 weeks of healing, and scarring is almost certain after 3 weeks.

Clinical evaluation of burn depth has its limitations due to different levels of experience of physicians hence being subjective. Although detection of very superficial or very deep burns is easier clinically, it is far less reliable for burns of intermediate depth. Another limitation is that the extent of tissue damage may not be immediate as dynamic changes during the post burn period, as well as infection, complicate the determination of burn depth and healing time, potentially resulting in the conversion of a superficial to a deep burn lesion.

Accurate assessment of burn depth is reflected on several aspects such as:

(a) Prognosis of burn patient.

(b) Rapid decisions taken in the early phase of acute burn in the emergency department such as fluid requirements, escharotomy indications and timing of referral.

(c) Assessment for treatment decisions on whether or not to operate and what to excise or leave on the actual burn wound.

(d) For research purposes, and for burn wound research that will aid in the development of novel treatments.

Our study included 64 patients with a mean age of 18.6, 71.9% (46) were males and 28.1% (18) were females. This was in contrast with the study conducted by Omar *et al*., which included 154 patients, 75.1% (116) were males and 24.9(38) were females with a mean age of 51 years[8].

In our study as well as studies of Adamzik *et al*., PFHb was measured by the gold standard spectrophotometric Harboe method where hemoglobin is quantified by measuring absorbance at three wave lengths (415, 380, and 450nm) and corrected by Allen's correction formula[9] in contrast to Damiani et al. used another spectrophotometric method using Drabkin reagent[10]. In contrast, also to Hodge et al. who used enzyme-linked immunosorbent assay (ELISA) to determine plasma free concentration[11].

In this study as well as Chung *et al*.,[12] and Hodge *et al*.,[11], blood samples were collected ethylenediaminetetraacetic acid (EDTA) blood collection test tubes and samples were centrifuged within 1–2h and stored at –70 to –80°C.

During the conduction of the study at handed it was decided to collect the blood samples for PFHb assays at baseline (0), 6, 12, and 48h from admission. In contrast to Hodge et al. who obtained their samples at baseline (0), 12, 18, and 24h of ECMO circuits[11].

It was reported that PFHb levels show a decline over 24h and return to baseline within 48h[13] so this guided our choice of timings of blood sampling. PFHb levels of patients in the study at hand peaked and reached highest values at 6h and tended to normalize at 48h suggesting that estimation of the PFHb levels may be more significant in the first 24h. PFHb concentrations are classified into normal values (<5mg/dl), moderate (5–50mg/dl) and severe (50mg/dl). All our patients showed moderate hemolysis with levels not exceeding 50mg/dl and no record of patients showing severe hemolysis. Burn depth was clinically evaluated as superficial or deep burn based on predominance of 80% of which type of burn, since burns usually present with different depths. It is valuable to mention that in this study, the total body surface area percentage and levels of PFHb on admission, at 6, at 12, and 48h of admission showed a moderately positive correlation.

CONCLUSION

Although a multitude of techniques and technologies exist to assess burn depth however clinical evaluation remains the most commonly used method. Examples of such technologies are laser doppler, dyes such as indocyanine green, thermography, histopathological assessment, magnetic resonance studies and hyperspectral imaging. These technologies showed promising results but were expensive, impractical, or time consuming.

Despite exponential increase in studies on burns in the literature, there is still controversy about the ideal method for assessing burn depth. An easy, quick, inexpensive method is yet to be devised and standardized.

In the treatment of acutely burnt patients, determining the depth of the burn wound remains a crucial and primary goal. Not only does depth determine a patient's prognosis, but it also determines the best clinical decision. As a result, determining the relative efficacies of different methods for determining burn depth remains a priority. Clinical evaluation is gold standard method for burn depth assessment yet is only accurate two thirds of the time at best. Intravascular hemolysis is a surrogate marker of thermal damage severity that is influenced by depth of burn as well as its extent. PFHb has the potential to be a useful diagnostic tool. This is the first laboratory test to show a link to depth of burn and total body surface area. It is suggested that the level of PFHb after burn injury is related to the depth and size of burn. This test can be a valuable diagnostic tool in burn assessment.

In light of the above, further work is needed to determine the clinical value of this test.

CONFLICT OF INTEREST

There are no conflicts of interest.

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