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## Cell free DNA as a biomarker in medicolegal assessment in burn patients

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**Abstract**---Background: Burn victims have higher levels of cell free DNA (cfDNA), which allows its use as a direct indicator of cellular damage and burn vitality. Aim: Determination of cfDNA levels in burn patients and their correlation with total body surface area burned percent (TBSA%). Subjects and methods: Burn cases were evaluated to determine the prevalence of age and sex variations, period of admission, TBSA%, and the etiology and manner of burns. The plasma cfDNA concentration was measured within 24 hours of the burn injury in 40 burn cases and 20 control subjects. Results: The mean age of the cases was 34.38 years (median 33 years). Most patients were males (62.5%). Burning by flame or scalding represented

50% of the cases. Accidental burns were the most predominant. The mean of admission periods was 36.55 days while the mean value of TBSA% of the cases was 16.68%. There was a statistically significant difference in cfDNA values between cases and control subjects ( $p = 0.001$ ). A positive correlation was found between cfDNA levels and TBSA% ( $r = 0.7$ ;  $p < 0.001$ ). Conclusion and recommendations: Levels of cfDNA were significantly different between burn cases and controls.

**Keywords**---burn, TBSA%, degree, cfDNA.

## Introduction

Burn injuries are among the most serious of all injuries and they constitute a major public health issue throughout the world. Burns are the fourth most prevalent type of trauma in the world, after traffic accidents, falls, and interpersonal violence. Burns are an underappreciated condition that can strike anyone at any time and in any location. Injuries due to friction, cold, heat, radiation, and chemical or electric damage can all cause burns, while heat from hot liquids, solids, or fire is the most common cause of burns [1,2]. Burns are the most painful and physically debilitating injuries, impacting nearly every organ system and resulting in high morbidity and mortality rates [3].

Regardless of the presence or absence of infection, severe burns cause proinflammatory immune responses in the peripheral blood and injured tissues. Trauma triggers inflammation and the production of damage associated molecular patterns in necrotic and wounded tissue, prompting the immune system to activate acute phase immune cells [4]. Cell free DNA (cfDNA) refers to DNA in the noncellular fraction of peripheral blood, which is composed of genomic (gDNA) and mitochondrial DNA (mtDNA). It is found in circulating plasma and other body fluids and originates mainly from apoptotic or necrotic cells [5].

Clinical diagnosis considering circulating cfDNA in plasma has been given attention recently. It is generally present in low levels, but it has been found to be increased in pregnant women as well as in patients with graft rejection, cancer, trauma, and stroke. After a burn injury, cfDNA is released into the bloodstream, which could be used as a helpful measure of total burn severity, depth, and surface area [6,7,8].

After burn and trauma injuries, the concentration of cfDNA in human plasma is frequently elevated. Injury results in two important activities: 1) destruction of parenchymal cells, and 2) indirect changes that occur in different circulating cells. According to several studies, cfDNA comes from both neutrophils (cNETs) and injured cells after burn injury and trauma [9]. This study evaluated whether cfDNA levels in burn patients have a relation to the TBSA%, type of burn, delay hours, and outcomes.

## Material and Methods

This was a prospective cross-sectional clinical study carried out at Kasr Alainy Burn Unit (KABU), Plastic Surgery Department in Kasr Alainy Hospitals, Faculty of Medicine, and Cairo University. Data were collected from patients' files, which included demographic data such as age, sex, residence, educational level, marital status, occupation, and medical history (manner of burn, etiology or cause of burn, and hours of delay and admission period in hours). Data were also obtained from medical examinations, which included the type and degree of burns and TBSA%. Blood samples were obtained [in ethylenediamine tetraacetic acid (EDTA) tubes] [10] from 40 burn cases within the first 24 hours after the burns occurred and from 20 control subjects. Levels of cfDNA in the blood samples were measured.

Patients who died within 24 hours of admission, who presented after 24 hours, had comorbidities such as cardiovascular diseases, infectious and inflammatory diseases, autoimmune disorders, cancers, pregnancy, age <18, chemical, electrical or irradiation burns, and those with concomitant trauma were excluded [11]. TBSA% was assessed and recorded using the Rule of Nines. Twenty healthy adult volunteers with their ages matching those of the patient group provided blood samples.

## DNA extraction and amplification

1. DNA was extracted using a QIAamp DNA Mini Kit (Catalog number: 51304) according to the manufacturer's instructions.
2. Plasma DNA for the  $\beta$ -globin gene, which is present in all nucleated body cells, was quantified using a real-time quantitative PCR technique. Primers were supplied by Thermo Fisher Scientific (sequences shown in Table 1) [12].

Table 1: The amplification primers sequence

|                                                         |
|---------------------------------------------------------|
| Beta-globin-354F (5'-GTG CAC CTG ACT CCT GAG GAG A-3'). |
| Beta-globin-455R (5'-CCT TGA TAC CAA CCT GCC CAG-3').   |

1. A 5' nuclease assay was used for amplification and product reporting [13]. The amplification reaction was linked to the release of a fluorescent reporter.
2. The Applied Biosystems StepONETM real-time PCR System was used to perform real-time quantitative PCR analyses (made in Singapore, 4375471). A typical analysis (blood centrifugation and DNA extraction followed by real-time PCR) took approximately three hours.

## Specimen collection

Venous blood (4 ml) was withdrawn from all participants and added to sterile EDTA vacutainers. Plasma was then separated within three hours by centrifugation at  $3,000 \times g$  for 10 minutes. Then the plasma supernatant was harvested and a second centrifugation session at  $14,000 \times g$  for 10 minutes was

done to ensure the full exclusion of any cellular element. Plasma samples were aliquoted and stored at  $-80^{\circ}\text{C}$  until the time of cfDNA extraction.

### Statistical analysis

The statistical package for the social sciences (SPSS version 26) was used to code the data (IBM Corp., Armonk, NY, USA). Data were analyzed as follows: Quantitative data were: 1) analyzed using the mean, standard deviation, median, minimum, and maximum; 2) compared using the nonparametric Kruskal–Wallis and Mann–Whitney tests [14]; and 3) analyzed for correlations using Spearman correlation coefficient [15]. P-values  $<0.05$  were considered statistically significant while  $p < 0.001$  was considered highly significant. Categorical data were: 1) analyzed using frequency (count) and relative frequency (percent); and 2) compared using a Chi-squared ( $\chi^2$ ) test. The exact test was used instead when the expected frequency was  $<5$  [16].

### Ethical considerations

The Research Ethics Committee of Cairo University's Kasr Alainy Faculty of Medicine approved the study (code MD-65-2019). Fully informed consent was obtained from the participants and their personal information was not disclosed.

### Results

The ages of the participants were 18–70 years (mean 34.38 years and median 33 years) with male predominance (62.5%). Most participants presented during winter season (40%). No statistically significant differences were observed in the sociodemographic data between patients and controls (Table 2).

Table 2: Sociodemographic characteristics of the studied groups

| Variables           | Cases             | Controls         | P     |
|---------------------|-------------------|------------------|-------|
| Age (mean $\pm$ SD) | 34.38 $\pm$ 13.88 | 34.05 $\pm$ 9.98 | 0.572 |
| Sex                 |                   |                  |       |
| Male (N/%)          | 25 (62.5%)        | 11 (55%)         | 0.576 |
| Female (N/%)        | 15 (37.5%)        | 9 (45%)          |       |
| Residence           |                   |                  |       |
| Urban (N/%)         | 24 (60%)          | 12 (60%)         | 1     |
| Rural (N/%)         | 16 (40%)          | 8 (40%)          |       |
| Occupation          |                   |                  |       |
| Non-worker (N/%)    | 14 (35%)          | 6 (30%)          | 0.832 |
| Mental (N/%)        | 12 (30%)          | 8 (40%)          |       |
| Manual (N/%)        | 7 (17.5%)         | 4 (20%)          |       |
| Student (N/%)       | 7(17.5%)          | 2 (10%)          |       |
| Marital State       |                   |                  |       |
| Married (N/%)       | 24 (60%)          | 13 (65%)         | 0.707 |
| Single (N/%)        | 16 (40%)          | 7 (35%)          |       |
| Educational         |                   |                  |       |
| Illiterate (N/%)    | 12 (30%)          | 5 (25%)          | 0.685 |
| Educated (N/%)      | 28 (70%)          | 15 (75%)         |       |

\*P- value is statistically significant < 0.05.

SD is standard deviation.

As shown in Table 3, accidental burns predominated (97.5%) and there were no homicidal cases. Burns caused by flame represented 50% and by hot liquids was 50%. The majority of cases (70%) presented with second-degree burns. The mean value of TBSA% was 16.68% (range 3%–38%). Healing occurred in 62.5% of the cases, while death occurred in 12.5% (Table 3).

Table 3: Manner, type, degree, Total body surface area burned percentage (TBSA%) and outcome in burn cases

| Clinical data               | Cases            |
|-----------------------------|------------------|
| Manner                      |                  |
| Suicidal (N/%)              | 1 (2.5%)         |
| Accidental (N/%)            | 39 (97.5)        |
| Burn type                   |                  |
| Dry (N/%)                   | 20 (50%)         |
| Scald (N/%)                 | 20 (50%)         |
| Degree                      |                  |
| First & second (N/%)        | 1 (2.5%)         |
| Second (N/%)                | 28 (70%)         |
| Second & third (N/%)        | 7 (17.5%)        |
| Third (N/%)                 | 3 (7.5%)         |
| First, second & third (N/%) | 1 (2.5%)         |
| Outcome                     |                  |
| Healed (N/%)                | 25 (62.5)        |
| Complicated (N/%)           | 10 (25%)         |
| Died (N/%)                  | 5 (12.5%)        |
| TBSA% (mean $\pm$ SD)       | 16.68 $\pm$ 8.62 |

SD is standard deviation.

(TBSA%) is Total body surface area burned percentage.

The mean value of delay was 7.88 hours with a range of 3–20 hours between the burn onset and presentation to the hospital. The mean stay at the burn unit was 36.55 days ranging from 16–65 days. Regarding the mean value of cfDNA, cases and controls showed highly statistically significant findings; that is, 1452.32 ng/ $\mu$ l and 95.90 ng/ $\mu$ l, respectively, with  $p < 0.001$ . The negative correlation between delay hours and cfDNA levels ( $r = -0.601$ ) was highly significant ( $p < 0.001$ ). The positive correlation between cfDNA and TBSA% ( $r = 0.7$ ) showed high significance as well ( $p < 0.001$ ).

High significance was observed between cfDNA levels and TBSA% for dry burns ( $p < 0.001$ ) and scald burns ( $p = 0.002$ ). Also, positive correlations were detected regarding cfDNA and TBSA% for dry burns and scald burns ( $r = 0.787$  and  $r = 0.647$ , respectively). A positive correlation was found for cfDNA levels and admission periods ( $r = 0.053$ ) with no statistical significance. As for the relation between cfDNA levels and patient outcomes, there was a statistically significant difference ( $p = 0.038$ ).

## Discussion

Thermal injuries are a common and severe acute traumatic event. In forensic science, determining the timing of a burn injury in a living person is critical [17]. In our study, accidental burns were the most predominant and no homicidal cases were reported. This was in agreement with studies by Nair and colleagues (2017) [18], Shahid and colleagues (2018) [19], and Mishra and colleagues (2017) [20]. An explanation for this finding is that most burns occur due to indoor domestic activities, especially for females, or in workplaces for males. Other causes were unsafe cookstoves, loose clothing, open flames used for heating and lighting, car accidents, accidental fires in homes, and gas explosions [20].

The current results showed that the majority of cases were second-degree burns. This was in agreement with Hashish and Abdel-Karim (2017) [21]. On the other hand, Wardhana and colleagues (2017) [22] and George and Abdellah (2017) [23] found that most burn cases presented with third degree burns and first-degree burns represented the lowest percentage of cases. This finding can be rationalized by the fact that most first and superficial second-degree burns are treated in outpatient clinics and do not require hospital admission [24].

In the current study, the mean number of hours of delay between the burn occurrence and presentation to KABU was 7.69 hours. This was in agreement with the studies done by Nair and colleges (2017) [18] and Sharma and colleges (2015) [25]. This finding may be explained by an urgent need for medical care, which depended on the magnitude of the injury, the initial response of the patient or relatives, and transport facility to the local hospital [2].

In the current study, the mean value of cfDNA levels in the first 24 hours after a burn occurrence was 1,452.32 ng/ $\mu$ l. In Hayun and colleagues (2019) [11] and Altrichter and colleagues (2010) [26], the mean values were 879 ng/ $\mu$ l and 678 ng/ $\mu$ l, respectively. This result is explained by the increase in cfDNA levels after burns, which are correlated to the burn severity, period of admission, and affected TBSA%. Studies have suggested that cfDNA levels can be used as a direct biomarker of cellular injury, which allows for a single, objective assessment of burn severity [9,11].

The present study revealed that there was a statistically significant difference in cfDNA levels between burn cases and controls, which was similar to results of other studies. Previous studies concluded that cases had significantly higher cfDNA levels than healthy controls, which is explained by necrosis from heat, degradation of neutrophils, and secondary damage from inflammation and hypoxia [8,11,27,28,29].

The current study showed a negative correlation between delay times and cfDNA levels, which was in agreement with Ren and colleagues (2013) [30] and Shoham and colleagues (2014) [28], who observed higher levels of cfDNA in the first six hours after trauma. The direct association between tissue injury in acute trauma and an increase in plasma cfDNA concentrations in the first several hours after the trauma, which was correlated with injury severity, was the basis for these findings. The spleen, liver, and kidneys appear to be involved in the clearance

mechanism of cfDNA. The half-life of fetal cfDNA was estimated to be 16 minutes. The first 24–48 hours after a severe burn are associated with the greatest degree of tissue damage so cfDNA levels could be a useful marker for the most severe and acute stages of the condition [30,31]

There was no statistically significant relation between burn types and levels of cfDNA in burn cases. On the other hand, it was found that cfDNA levels showed a significant relation with burn types and high levels of cfDNA were observed in dry burn cases. This can be explained by deeper burns and more tissue destruction being found in dry burns [8].

The present study revealed a statistically significant correlation between cfDNA levels and TBSA% values in dry burns and scald burns. Other studies reported that cfDNA levels were correlated with TBSA% values [26,27,28]. However, Chiu and colleagues (2006) [8] showed that cfDNA levels were correlated with burn surface areas in scald burns but not in dry burns. On the other hand, Sharon and other researchers (2020) [32] concluded that there was no significant relation between cfDNA levels and TBSA% values. These results could infer that the production of cfDNA from cellular death, apoptosis, and necrosis increases with higher TBSA% involvement [33].

The present work showed a positive correlation between cfDNA levels and admission periods, which agreed with some previous studies [11,32]. On the contrary, some studies concluded that there was no significant relationship that could be explained by greater burn severity causing a higher level of inflammation and increased cell death with more cfDNA released and detected [28,34]. The current study showed a statistically significant difference between cfDNA levels and outcomes. Similarly, Altrichter et al. 2010 [26] and Shoham et al. 2014 [28] reported that there were significant differences between survivors and nonsurvivors, who had higher levels of cfDNA. The rationale of this finding was cfDNA elevation in burn cases is related to the period of admission and TBSA%, and all of these factors have a strong correlation with outcomes and mortality rates [11].

## **Conclusion**

As a conclusion, this study confirmed the increase in cfDNA levels in burn patients, which may be caused by cell damage and apoptosis. Therefore, cfDNA is related to TBSA%, delay hours, and outcomes and it may serve as a valuable biomarker for burns. However, further studies should be designed with more than one sample taken over several hours to assess changes cfDNA levels according to delay times and the measurements of cfDNA levels may be helpful in dating the burn.

## **Declaration of Conflicting Interests**

The authors declare no conflicts of interest.

## Ethical approval

The Research Ethics Committee of Cairo University's Kasr Alainy Faculty of Medicine gave its approval to the study (code MD-65-2019). A fully informed consent was used and participants' personal information was not disclosed.

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