Changes in serum phosphorus level in patients with severe burns: A prospective study

Siamak Rimaz a, Anoush Dehnavi Moghadam b, Mohamadreza Mobayan c, Mehdi Mohammadi Nasab b, Sheyda Rimaz d, Roghayeh Aghevari e, Zakiyeh Jafaryparvar c,⁎, Enayatollah Homaei Rad f

a Anesthesiology Research Center, Guilan University of Medical Sciences, Rasht, Iran
b Guilan Road Trauma Research Center, Guilan University of Medical Sciences, Rasht, Iran
c Razi Clinical Research Development Center, Guilan University of Medical Sciences, Rasht, Iran
d Student Research Committee of Anzali International Campus, Guilan University of Medical Sciences, Rasht, Iran
e Guilan University of Medical Sciences, Rasht, Iran
f Guilan Road Trauma Research Center, Guilan University of Medical Sciences, Rasht, Iran

Article history:
Accepted 26 March 2019
Available online xxx

Keywords:
Burn
Phosphate
Hypophosphatemia
Supplementation

ABSTRACT

Background: The second most abundant mineral in the body, phosphorus (P), is absorbed in the small intestine after ingestion enhanced by 1,25-dihydroxy vitamin D, and its excretion is exclusively regulated by the kidney. It is clinically significant, aside from its disturbance in burn ICU patient’s P mechanism. The increasing rate of morbidity and mortality among the patients can be associated with severe hypophosphatemia. The current study aimed at investigating the changes in serum P levels in the early period after burns, the relationship between serum P level and TBSA (total body surface area) of burn, and the impact of hypophosphatemia on patients’ clinical outcomes.

Material and methods: The current prospective, observational study was conducted on 137 patients hospitalized in the burn intensive unit (ICU) of Velayat Sub-speciality Burn and Plastic Surgery Center from December 2015 to May 2017. According to the TBSA percentage, the patients were divided into three groups. The level of serum P was determined in the 1st, 3rd, 5th, 7th, and 9th days of hospital stay and before discharge. To evaluate the trend of P changes in the sixtime-points, the average changes along with 95% confidence intervals (CI) were used for multivariate analysis of variance with repeated measures (repeated measures ANOVA). A P-value of 0.05 or less was considered statistically significant. The analyses were performed using SPSS software, version 19 (SPSS Inc.).

Results: Totally, 137 patients (70% male, mean age 32 ± 21 years, and TBSA 32.6 ± 14%) were included in the study. The overall incidence of hypophosphatemia was 75.1%. Hypophosphatemia developed as early as 1.66 ± 0.136 (95%CI: 1.4—1.9) days after injury. The highest decrease in the serum P level was observed on the 3rd and 5th days after burn as 2.78 mg/dL and 2.85 mg/dL, respectively (P-value = 0.001). A correlation was observed between TBSA and serum P level. The mean serum P level decreased with increasing the percentage of burns. There was a correlation between serum P level and mortality; therefore, a decrease in serum P level increased the patient's mortality rate (P < 0.05).

Conclusion: The current study highlighted that hypophosphatemia is often observed in patients with burn injuries during their hospitalization. It is potentially beneficial to identify patients at risk of hypophosphatemia. Therefore, it is suggested that P level be assessed regularly in patients with burn injuries for the timely initiation of P replacement therapy.

⁎ Corresponding author at: Razi Hospital, Razi Clinical Research Development Center, Guilan University of Medical Sciences, Sardar Jangal Street, Rasht, 4144895655, Iran.
E-mail address: jafaryparvar_zakiyeh@yahoo.com (Z. Jafaryparvar).
https://doi.org/10.1016/j.burns.2019.03.015
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Please cite this article in press as: S. Rimaz, et al., Changes in serum phosphorus level in patients with severe burns: A prospective study, Burns (2019), https://doi.org/10.1016/j.burns.2019.03.015
1. Introduction

The second most abundant mineral in the body, phosphorus (P), is absorbed in the small intestine after ingestion, enhanced by 1,25-dihydroxy vitamin D, and its excretion is exclusively regulated by the kidney. P accounts for approximately 1.1% of the total body mass, approximately 85% of which is found in the skeleton in the form of calcium P. A significant portion of the body’s non-skeletal P stores resides within skeletal muscle either as free inorganic P (Pi) or as bounded within high-energy P molecules (HEP) including ATP, adenosine diphosphate (ADP), adenosine monophosphate (AMP), or phosphocreatine (PCr). As a principal substrate of ATP synthetase, Pi obviously plays a critical role in maintaining ATP production [1].

It is clinically significant, aside from its disturbance in burn ICU patient’s P mechanism [2]. Burn injuries may lead to a progressive reduction in the P level, with the lowest amounts between the 2nd and 5th days after burn; thus, additional supplemental P should be regarded in this period. Despite the aggressive prescription of supplemental P in patients with massive burn injuries, serum P levels rarely return to normal range before the 10th day after burn [3]. The carbohydrate administration, respiratory alkalosis, and diuresis of edema fluid are several factors that alter the serum P level after a burn injury and are responsible for hyperphosphatemia in the early period after burns [4]. This hypothesis was recently supported by researchers in a large series of patients with burns [5]. Moreover, hypermetabolism is a hallmark of stress response to burns, and skeletal muscle ATP turnover is elevated after burn trauma, owing largely to greater protein synthesis and breakdown rates [1]; ultimately, ATP is depleted in muscles of patients with burn trauma [6,7]. It is noteworthy that during the tissue depletion of P along with hypophosphatemia, as a simultaneous attempt to conserve P by the body, a marked reduction occurs in the two hormones that facilitate P excretion; i.e., parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23) in children after a severe burn injury (Fig. 1). FGF23 is a bone-derived hormone involved in P and vitamin D (VD) regulation [8].

Hypophosphatemia is typically categorized as mild (serum P concentration of 2.5–2.9 mg/dL), moderate (1.2–2.4 mg/dL), or severe (<1 mg/dL). Although mild to moderate hypophosphatemia is usually asymptomatic, severe hypophosphatemia can be potentially associated with significant morbidity [2]. Up to 30% mortality is reported in patients with severe hypophosphatemia [9]. Clinical manifestations of hypophosphatemia are extraordinary unless the serum P level reaches below 1 mg/dL. Severe skeletal muscle deficiency and respiratory failure secondary to diaphragmatic weakness may occur [10–12]. Central nervous system (CNS) disorder includes lethargy confusion, and gait perturbation. Hematologic manifestations including acute hemolytic anemia and leukocyte dysfunction may occur. Cardiovascular manifestations include acute LV (left ventricular) dysfunction and creation of reversible dilated cardiomyopathy that typically responds only to P supplementation; furthermore, rhabdomyolysis may happen [13]. In the condition with severe hypophosphatemia (P level <1 mg/dL), treatment should be started with parental P. In moderate hypophosphatemia (P level 1–2.4 mg/dL), oral P can be prescribed in patients capable of taking medications orally or via a nasogastric tube [2]. Hypophosphatemia is reported in up to 5% of hospitalized patients and 30%–50% of patients with septic shock or the ones hospitalized in intensive care units (ICUs) [14–17]. The reported incidence of hypophosphatemia in burn patients varies from 0.2% to 2.2% for all admitted patients, but it can be 21.5% or even higher in some patients with higher percentages of TBSA (total body surface area) burn [18].

In clinical practice, it is observed that hypophosphatemia is a common problem in patients with severe burn injuries in burn centers. Since the study of hypophosphatemia in the early period after burns is not performed in the newly established burn center, Velayat Sub-specialty Burn and Plastic Surgery Center in Guilan Province, Iran, the current study aimed at investigating the serum P level changes in the early period after burns, the relationship between serum P level and TBSA of burn, and the hypophosphatemia impact on patients’ clinical outcomes.

2. Material and methods

The current prospective, observational study was conducted in the burn intensive care unit (BICU) of Velayat Sub-specialty Burn and Plastic Surgery Center, Guilan Province, to determine the changes in serum P level in the early period after burns, the correlation between serum P level and TBSA burn, and the impact of hypophosphatemia on patients’ outcomes. The current study was approved by the Ethics Committee of Guilan University of Medical Sciences (GUMS) (ethical code: IR.GUMS.REC.1395.48). Written informed consent was taken from all patients or their legal guardians in the presence of a witness. Then, they were included in the study.

Velayat Sub-specialty Burn and Plastic Surgery Center is the only burn center in Guilan Province (the North of Iran) that admits patients with burns from Guilan and other provinces.

All adult patients (age >18 years and TBSA ≥20%) admitted to the ICU from December 2015 to May 2017 were included in the study. The percentage composition obtained less than 20% in patients with BURNS, pediatric patients, patients that died within 24 h of after burn injury, patients with concurrent medical conditions such as chronic renal diseases and severe malnutrition, and patients faced explosion followed by brain injury (since brain trauma may disrupt the signaling pathway or reduce P excretion pathway activity) were excluded from the study [1]. All patients were resuscitated using the Parkland formula with lactated Ringer solution at 4 mL/kg% TBSA to maintain minimum arterial pressure ≥65 mmHg and urine output of at least 0.5 mL/kg/h. The early intubation along with mechanical ventilation was performed after monitoring the acute respiratory distress symptoms due to upper airway obstruction or inhalation injury. Serum P levels were measured by an autoanalyzer (Hitachi Automatic Analyzer, Furuno-CA185-JAPAN) in the 1st, 3rd, 5th, 7th, and 9th days after burn and before discharge in patients admitted to BICU. Standard
levels of serum P reach prior to the tenth-day after burn following aggressive P supplementation; consequently, the final confirmation was performed on the 9th day. Normal plasma P levels range from 3.0 to 4.5 mg/dL. Hypophosphatemia was defined by a plasma P level of less than 3.0 mg/dL, and patients were classified into three groups [mild (2.5–2.9 mg/dL), moderate (1–2.4 mg/dL), and severe (<1 mg/dL)], according to the minimum P level within the 10 days of burn injuries based on previously mentioned classification. When the levels recede below 2.0 mg/dL, intravenous P repletion initiated regularly with solutions of sodium or potassium P containing 0.08–0.16 mMKg body weight (2.5–5 mg/kg body weight) of elemental P over six hours. For mild asymptomatic hypophosphatemia (serum P 2.3 mg/dL), 5 mL of Phospho-Soda®-soda containing 4.2 mM/mL of elemental P was regularly administered three times daily. Early enteral administration was started within 48 h after burn and patients were operated in the 3rd day after burn if thermodynamically stable. Wound excision was completed within five days after burning.

According to the percentage TBSA burned, the patients were divided into three groups: Group A: 20%–39% TBSA burned; Group B: 40%–59% TBSA burned; and Group C: more than 60% TBSA burned. Demographic profiles were recorded including age, gender, height, weight, burn size, alcohol consumption, time to develop hypophosphatemia, past medical history, and concurrent medications. In the current study, serum P levels were measured within the time from injury to determine the alterations in the early period after burn, the relationship between serum P level and TBSA, and the impact of hypophosphatemia on patients’ clinical outcomes.

Data were described as means (standard deviation) for quantitative variables, and absolute (relative) frequencies for qualitative ones. The normal distribution of quantitative variables was evaluated using the Kolmogorov–Smirnov test. Correlation between variables was assessed using the Chi-square, ANOVA, or Kruskal–Wallis tests (in the absence of data). The average changes along with 95% confidence intervals (CI) were used for multivariate analysis of variance between/n repeated measurements (repeated measures ANOVA) to evaluate the trend of P changes in the six time-points. The compound symmetry assumption was studied with the Mauchly sphericity test. In this case, paired comparisons (using the least significant difference method) were used to determine a significant difference between repetitive sizes or interpersonal variables. The serial serum P level changes were analyzed in all patients after burn injuries. In this case, after observing the significance of Mauchly sphericity test and the lack of spherical or composite symmetry in repeated measurements of serum P levels, the Pillai trace multivariate test was used to compare the changes in serum P levels in six measurement periods and indicate a significant difference between the adjusted means of the four measurement periods. Logistic regression was employed to examine the relationship between serum P level and mortality after controlling variables including age, gender, burn severity, and burn reason.

A P-value of less than 0.05 was considered statistically significant. Data analyses were performed with SPSS software, version 19 (SPSS Inc.).

3. Results

Totally, 137 patients (70% male, mean age 32 ± 21 years (range 18–91), and TBSA 32.6% ± 14%) were examined prospectively (Table 1). Within nine days after injury and the day before discharge, all patients’ serum P levels were evaluated. Of 137 patients admitted to Velayat Center, 80 (59%) were male. According to the TBS, 72 (53%) patients were placed in Group A, 29 (21%) in Group B, and 36 (26%) in Group C. No significant differences were observed in gender between the three groups of TBSA burn (results not shown). Patients’ demographic characteristics are described in Table 1. The current study findings indicated that 34 (24.8%) patients did not have hypophosphatemia and 103 patients (75.2%) developed hypophosphatemia, of whom 20 (19.4%) had mild hypophosphatemia, 80 (77.6%) moderate hypophosphatemia, and three (3%) severe hypophosphatemia; consequently, the overall prevalence of hypophosphatemia in the current study was 75.1%. Hypophosphatemia was developed as early as 1.66 ± 0.136 (95% CI: 1.4–1.9) days after injury.

The serial serum P level changes were analyzed in all patients after burn injuries. According to the significance of Mauchly sphericity test (P = 0.001) and the lack of spherical or compound symmetry in repeated measurements of serum P levels, the Pillai trace multivariate test was employed. In a matched-pair comparison of the mean serum P levels, there was a significant difference between the measurement times (P-value = 0.001), except for the 3rd and 5th days. The highest decrease in serum P level was observed on the 3rd and 5th days after burn as 2.78 and 2.85 mg/dL, respectively (P-value = 0.001) (Fig. 2).

The patients were divided into three groups according to TBSA burn, and the changes in serum P levels were analyzed at each time point for each group (TBSA 20%–39%, n = 72; TBSA 40%–59%, n = 29; TBSA ≥60, n = 36). The mean serum P levels decreased three to five days after burn in groups B and C (Fig. 3). There was a correlation between TBSA burn and serum P level; therefore, an increase in the burn percentage decreased the mean serum P level; consequently, patients with TBSA ≥60% had lower serum P levels compared to other groups (A and B). In each period, there was no significant difference in the mean serum P levels between the groups classified according to TBSA burn (Fig. 3).

The relationship between serum P level and mortality in patients with burn injuries is shown in Table 2. As indicated in the table and according to the logistic regression, with a decrease in the serum P level, the patients’ mortality rate increased and the correlation was statistically significant (P < 0.05).

In the end, multivariable logistic regression analysis was employed to investigate the relationship between serum P levels and hospital mortality. In this regression model, variables such as age, severity and cause of burn, and serum P levels were considered as independent variables and the binary variable (duality) of mortality was considered as a dependent variable in the model (Table 2).

Table 1 – Demographic and clinical characteristics of study population.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (N = 137)</th>
<th>% (100.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>32 ± 21 years (18–91 years)</td>
<td>70%</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>96</td>
<td>70%</td>
</tr>
<tr>
<td>Female</td>
<td>41</td>
<td>30%</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.5 ± 6.4</td>
<td></td>
</tr>
<tr>
<td>Cause of injury</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flame, n (%)</td>
<td>45</td>
<td>33%</td>
</tr>
<tr>
<td>Scald, n (%)</td>
<td>42</td>
<td>31%</td>
</tr>
<tr>
<td>Electric, n (%)</td>
<td>4</td>
<td>3%</td>
</tr>
<tr>
<td>Explosion</td>
<td>46</td>
<td>34%</td>
</tr>
<tr>
<td>Percent TBSA burned, mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group (A) &lt;40%</td>
<td>72</td>
<td>53%</td>
</tr>
<tr>
<td>Group (B) =40–60%</td>
<td>29</td>
<td>21%</td>
</tr>
<tr>
<td>Group (C) &gt;60%</td>
<td>36</td>
<td>26%</td>
</tr>
<tr>
<td>Inhalation injury</td>
<td>41</td>
<td>29%</td>
</tr>
<tr>
<td>Need to mechanical ventilation</td>
<td>29</td>
<td>21%</td>
</tr>
<tr>
<td>ETOH</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>
The serum P level changes were compared between the survivors and non-survivors and the results indicated a significant decrease in the mean serum P level from the 3rd to 5th days after burn in the two groups. The serum P level rose steadily in survivors and returned to the normal range within a short time after the 5th day. In this period, in patients that died, the serum P level progressively remained low and somewhat increased following the 8th and 9th days; however, it never returned to normal in these patients. In this period, the mean serum P level was significantly lower in non-survivors than in survivors (P = 0.037) (Fig. 2).

4. Discussion

Velayat Sub-specialty Burn and Plastic Surgery Center is the exclusive burn center in Guilan Province. The current study aimed at determining the serum P level changes in the early period after burn, the relationship between serum P level and TBSA, and the effect of hypophosphatemia on patients’ outcomes in the burn center. It was the first study conducted in Velayat Center to investigate the change of serum P levels in patients with burn injuries and its correlation with TBSA.

Burn injuries lead to a progressive reduction in the P level [3], and this condition is recently supported by the authors in a large series of patients with burn injuries [9]. Hypophosphatemia is reported in hospitalized (up to 5%), alcoholic (up to 28–50%), and septic shock or ICU admitted patients [14–16,19]. In the current study, the number of alcoholic patients was nil. The possible explanation for this finding is the prohibition of alcohol consumption in Iran. Considering religious beliefs and the current laws in Iran, alcohol consumption is considered a crime and is prohibited. The setting of the current study was a governmental center and despite that the questionnaire had an item related to the history of alcohol consumption, all patients’ responses to this question were negative. Thus, there was no evidence of a history of alcohol consumption among patients. In a prospective study, hypophosphatemia was common in critically ill children (prevalence of 62%) during the 1st seven days of PICU (pediatric intensive care unit) admission [13]. Loven et al., found that the incidence of hypophosphatemia in patients with burn injuries varied from 0.2% to 2.2% for all admitted patients, but it reached 21.5% or even higher in certain patient groups such as the ones with higher percentages of TBSA burn [18]. Loghmani et al., reported a higher incidence of hypophosphatemia in patients with burn injuries (75.6% of patients with TBSA ≥40% in the 3rd day after burn) [20]. In the study by Yang et al., the overall incidence of hypophosphatemia was 64.7% [21]. Leite et al., in a recent study reported the incidence of hypophosphatemia as 79.5% in children with severe burn injuries [22].

Only one case report described late-onset of hyperphosphatemia in a patient with severe burn injury [23]. However, hyperphosphatemia is less frequently addressed. Kuo et al., in a prospective study on 301 patients with burn injuries based on the serum P level on the admission day, reported 52 patients with hyperphosphatemia (17.2%). The data were mostly obtained within 24 h after the burn injury due to high medical accessibility in Taiwan, and they explicated that hyperphosphatemia is associated with higher TBSA burn. The early detection of hyperphosphatemia and its correlation with higher TBSA burn may reflect the severity of tissue injury and the subsequent release of P within the intracellular compartments [24].

In the current study, the overall incidence of hypophosphatemia was 75.1% in patients with TBSA ≥20%. Compared to the results of previous studies, the incidence of hypophosphatemia in patients with burn injuries under the current study was higher than that of Loven et al. [18], but it was consistent with the results of studies by Loghmani et al., Yang et al., and Leite et al. [20–22]. The possible reason for the high incidence of hypophosphatemia in the current study may be due to the lack of a prevention standard protocol in the burn center, in addition to the major mechanisms for the creation of hypophosphatemia in patients with severe burn injuries, including decreased intestinal absorption, internal redistribution, increased urinary loss, and the high catabolic status.

The patients’ lowest serum P level typically happens 3–5 days after burn; throughout the period of edema mobilization, despite both standard and aggressive P supplementation, the normal level of serum P rarely reaches the prior level until the 10th day after burn [3]. Yang et al., reported that serum P levels decreased significantly during 3–5 days after burning. The mean ± SD of serum P level was 1.8 ± 0.9 mg/dL, 3–5 days after burn [21]. Loghmani et al., reported that serum P levels in patients of the group C (≥40% TBSA) decreased over the 3rd, 6th, and 9th days after burn [20]. According to the current study results, hypophosphatemia developed as early as 1.66 ± 0.136 days after injury and the pattern of changes in serum P was
similar to the ones reported in previous studies. The highest decrease in serum P level was observed on the 3rd and 5th days after burning. The serum P level changes were evaluated from baseline to the 3rd day by Kuo et al., demonstrating hyperphosphatemia at baseline in patients with burn injuries when admitted to the burn unit; they found mortality rates independent of the TBSA burn, inhalation injury, and APACHE II score; moreover, among patients surviving longer than three days, the serum P levels significantly decreased from the baseline to the 3rd day [21,24–26].

Loghmani et al., reported that the incidence of hypophosphatemia increased with TBSA burn and the mean serum P levels on the 3rd, 6th, and 9th days after burn increased in patients with 20%–29% TBSA; however, the mean serum P levels in patients with ≥40% TBSA decreased at these intervals [20]. Nevertheless, Yang et al., found no significant difference in the mean serum P level according to TBSA burn [21]. In the current study, the mean serum P level in the patients of group A did not decline on the 1st, 3rd, and 5th days after burn, although in the patients of groups B (40%–59% TBSA) and C (>60% TBSA), it decreased in this period. No significant difference was observed in the mean serum P levels between the groups, according to TBSA burn in each interval. The reasons for this difference can be due to inadequate physiological compensation of hypophosphatemia despite the initiation of treatment with supplemental P in patients with major burn injuries.

It remains unclear whether hypophosphatemia contributes to mortality or it is merely a marker for the severity of illness. Multiple studies showed an association between hypophosphatemia and increased mortality [19,27–30]. However, hypophosphatemia was not associated with increased mortality due to cardiac surgery [27] and diabetic ketoacidosis [30]. Severe hypophosphatemia (<1.0 mg/dL) is associated with significant morbidity and a four-fold increase in mortality [9,31,32]. However, Yang et al., found no significant difference in serum P levels between survivor and non-survivor patients with burn injuries [21].

To evaluate the effect of hypophosphatemia on survival, the relationship between serum P levels and burn mortality rate was analyzed, which showed that a decrease in serum P level significantly increased the mortality rate. The results of the current study were similar to those of previous studies, which confirmed a positive relationship between the low serum P level and increased mortality rate [19,27–30]. However, a study conducted by Yang et al., highlighted no correlation between mortality and hypophosphatemia [21]. Katayama in a retrospective cohort study showed that the mortality of patients with severe hypophosphatemia was high, but the lowest level of serum P was not associated with in-hospital mortality; they suggested that what directly affected mortality in such patients is not the level of hypophosphatemia itself, but the intensity of prime diseases causing hypophosphatemia [33]. Despite the significant relationship between severe hypophosphatemia and mortality in patients with burn injuries, the role of other factors inducing death should not be neglected.

Comparison of the serial serum P level changes in survivors and non-survivors showed that the level decreased in both groups of patients between the 3rd and 5th days after burn, but in the survivors, the response to supplemental P was adequate, and the serum P level rose steadily after the 5th day to eventually reach the normal range; however, in non-survivors, the serum P level after the 5th day was still low, despite treatment with oral and parenteral P, and only after the 8th and 9th days, it raised, but not reaching the normal range. This finding was contrary to that of Yang et al. They found that following the initial reduction in the serum P levels between the 3rd and 5th days after burn, the level of P steadily rose both in survivors and non-survivors [21]. Rousseau et al., based on a study in adult patients with burn injuries, revealed a progressive increase in FGF23 levels after burn, indicating the potential role of FGF23 in burn-related hypophosphatemia [34]; nonetheless, in a recent analysis, FGF23 did not increase, which disputed their previous assumption [35]. Conceivably, the reasons for the progressive decrease in non-survivor patients’ P levels are major burn injuries and the inability to physiologically compensate, as well as the inability to start enteral P during the 1st days after burn, and the lack of standardized guidelines for parenteral P replacement therapy with massive burn injury in Velayat Center.

The analysis of de-identified data from 181 pediatric patients with ≥20% TBSA burn indicated acute hyperphosphatemia (<3.5 mg/dL) with a mean of 2.2 ± 1.4 following admission in 49% of the patients. Serum P recovered to normal after a mean of 4.7 ± 5 days (ranging 1–15 days) [36]. It is noteworthy that during the increase in intracellular demand for P and some overall reduction in tissue P, there is a marked reduction in the two hormones that facilitate P excretion, i.e., parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF 23). Accordingly, the possible decrease in PTH and FGF23 may be crucial to the adequate preservation of P amounts to maintain the increased intracellular P utilization. Unfortunately, the current study could not measure the serum levels of PTH and FGF 23 in the two groups of survivors and non-survivors to determine whether, similar to those of the children with burn, the two principal P uric hormones in the body were suppressed after severe burn injury or in adult patients with burn injuries, these changes occurred differently to maintain the normal range. The sample size and conducting the study in a single burn center are the only drawbacks of the current study. Despite these limitations, it is believed that these preliminary findings can assist further studies in the local population. It is important to investigate whether hypophosphatemia causes higher mortality itself or it is associated with higher severity of illness.

### 5. Conclusion

The current study results showed that hypophosphatemia frequently developed in patients with massive burn injuries, which can lead to several physiological alterations in cellular function.

It is potentially beneficial to identify patients at risk of hypophosphatemia; therefore, it is suggested that P levels be routinely measured in BICU admitted patients and prompt P replacement be conducted when hypophosphatemia was recognized in order to minimize any sequelae of this potentially deleterious electrolyte deficiency in patients with massive burn injuries.
Funding

This research was financially supported by the vice-chancellorship of research of Guilan University of Medical Sciences.

Conflict of interest

The authors declare no conflict of interest.

Author’s contributions

Siamak Rimaz: design the study, writing of the article, reading and approving the final version of the article before submission.

Anoush Dehnavi Moghadam: design the study, writing of the article, reading and approving the final version of the article before submission.

Mohammadreza Mobayan: design the study, writing of the article, reading and approving the final version of the article before submission.

Mehdi Mohammadi Nasab: design the study, collecting data, writing of the article, reading and approving the final version of the article before submission.

Shayda Rimaz: design the study, collecting data, writing of the article, reading and approving the final version of the article before submission.

Roghayeh Aghabati: design the study, collecting data, writing of the article, reading and approving the final version of the article before submission.

Zakiyeh Jafariparvar: design the study, writing of the article, reading and approving the final version of the article before submission, submitting the manuscript.

Enayatollah Homaie Rad: analysis and interpretation of data, writing of the article and revision its content, reading and approving the final version of the article before submission.

Acknowledgements

This study was extracted from the anesthetist specialty student dissertation. The researchers would hereby like to thanks to all the patients who participated in the study, their families, and the nurses of B.I.C.U who offered their great assistance.

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