Assessment of Neopterin and Interleukin-1 Beta Serum Levels in Burn Patients

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Abstract

Background: The burn is an injury consisting of the destruction of the skin and the underlying tissues. The neopterin(Neo) is produced by activated macrophages, in response to interferon-gamma derived from activated T cell. Interleukin1 beta (IL-1β) is an important mediator of the inflammatory response.

Objective: Estimation of Neo and IL-1β in burned patients to determine the changes in these parameters in relation to percentage of total burn body surface area (TBSA%) and the duration of hospital stay.

Patients and Methods: Fifty burned patients who were admitted to West Erbil Emergency Hospital in Erbil governorate were included. Out of 50 burnt patients 20 patients were secondly sampled to follow-up serum levels for Neo and IL- β. The burn patients in this study was divided into four groups according to the TBSA%.

Results: Comparing mean concentration of serum Neo and IL-1β in burn patients and healthy control revealed increased levels of Neo and IL-1β in burnt patients with increased TBSA% , indeed IL-1β serum level also increased in non-survivor burn patients compared with survivor patients and HC. Indeed levels of Neo level increased significantly in (10) day post burn.

Conclusion: Levels of Neo and IL-1β increased in burnt patients with increased TBSA%, non-survivor compared with survivor patients and HC. Indeed levels of Neo level increased in (10) day post burn.

Key words: Neopterin, Interleukin1 beta , total body surface area, burn patients.

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Introduction

A burn is an injury to the skin or other organic tissue principally caused by heat, radiation, radioactivity, electricity, friction or contact with chemicals [1]. The extent of a burn is expressed as the total percentage of body surface area (TBSA%) and very helpful as guide management. At the site of injury, cytokines and inflammatory mediators release will cause systemic effects once the burn covers 30% TBSA [2]. Suppression of the innate and adaptive immune system as a result of systemic inflammation lead to sepsis, wound healing complications, multi-system organ failure [3].

Production of pro-inflammatory cytokines such as interleukin-1 beta (IL-1β), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) and prostaglandin E2 (PGE2) from innate immune cells show a progressive increase however counter-inflammatory responses such as interleukin-10 (IL-10) and transformer growth factor

beta (TGF-β) produced from cells of the adaptive immune system [4]. The uncontrolled release of pro- and anti-inflammatory cytokines promotes immunological dysfunction and significant systemic inflammation, which result in tissue damage, multiple organ failure, or death in burn patients [5]. Activated macrophages and monocytes released neopterin, a specific marker of innate immunity. Neopterin has been used a large number of studies as a marker of IFN activation [6]. Macrophages, monocytes, fibroblasts, and dendritic cells, produce both IL-1α and IL-1β. They form an important part of the inflammatory response of the body against infection [7].

The aims of this study was estimation serum levels of Neo and IL-1β in burn patients regarding to TBSA% area and survivor state.

Patients and Methods

Fifty burned patients who were admitted to West Erbil Emergency Hospital (WEEH) in Erbil governorate were included in this study from February 2012 - April 2012. Out of 50 burnt patients; 20 patients were secondly sampled to follow-up their immune profile .The mean age of patients was 22.23 ± 1.48; ranged from 1 year to 49 years old. The total number of burnt males and females were 18 (36%) and 32 (64%) respectively. The mean of TBSA burned were 35.03 ± 2.89 and ranged from 1% - 86%. Twenty healthy individuals composed of 5 (25%) males and 15 (75%) females. Their mean ages were 19.73 ± 2.10 had been chosen from the staff of WEEH in Erbil governorate who apparently healthy. Blood samples were collected from burned patients, and transferred into ten milliliters (10 mls) sterile tubes let to be a clot in order to separate a serum and centrifuged at 3000 rpm for three minutes and serum was separate from whole blood and stored in deep freeze (-70°C) in aliquot into several Eppendorf tubes until assayed. Kits were used in the study, Neo ELISA kit/ LDN/ Germany and IL-1ß ELISA kit/ Raybiotech/ U.S.A.

This study was approved by the research ethics committee of Hawler Medical University /College of Medicine, Erbil. All participants were provided with written informed consent after the study purpose and procedures were explained.

Statistical analysis

Statistical analysis was done by using Statistical Package for Social Sciences version 19.0. Cytokines(Neo and IL-1β) concentrations will be expressed as means ± SE. For comparisons between two groups, we determined the significance of differences between means by t-tests. Comparisons between multiple groups were performed by ANOVA.

Results

The burn patients in this prospective study was divided into four groups according to the percentage of total body surface area (TBSA%) burned carry out on (50) burn patients admitted to burn unit in West Erbil Emergency Hospital (WEEH) in Erbil governorate and (20) apparently healthy non burn individuals who regarded as healthy control group (HC). On the basis of TBSA % , burned patients were categorized into 4 groups (G1,G2,G3 and G4). The highest number of burn injury located in second group (G2) 23 (46%), with TBSA (26-50%), in which the female were higher than male as shown as in (Table 1). Table 2 shows no significant differences in mean concentration of serum (Neo and IL-1β) in burn patients and healthy control (P>0.05) .

Table (1): Burn patients groups according to the TBSA%

Whole groups	Total No.(%)	Male No.(%)	Female No.(%)	Age (year) Mean	TBSA % Mean
G1	18(36)	9(47)	9(30)	2.4 - 44 (18.08)	6-25 (16.67)
G2	23(46)	7(41)	16(48)	1.2 - 39 (22.49)	26-50 (37.28)
G3	6(12)	1(5)	5(15)	20 - 36 (28.17)	51-75 (59.17)
G4	3(6)	1(5)	2(6)	16 - 38 (26.33)	76-100 (86.67)
Total	50(100)	18(100)	32(100)	0-50 (22.23)	1-86 (35.03)
G1: (6-25%); G2: (26-50%); G3: (51-75%); G4: (76-100%)					
TBSA: total body surface area					

Table (2): The descriptive statistics for the mean concentration of serum (Neo and IL-1β) in burn patients and studied groups.

Biomarkers	Burn patient No.50	Healthy control No.20	P-value (probability) t-test
	Mean ± SE	Mean ± SE	
Neo (ng/ml)	10.89 ± 2.01	15.5± 2.288	0.138(NS)
IL-1β(pg/ml)	0.67 ± 0.25	0.188± 0.018	0.063(NS)
NS= Non-Significant (P>0.05)			

Meanwhile, regarding the estimation of mean serum concentration of neopterin in studied groups, (Table 3) revealed highly significant differences (P<0.01), when comparison conducted between mean serum concentration of Neo belong to different burn patient groups (G1), (G2), (G3), (G4) and HC. Significant differences also revealed comparing all groups versus G4 and HC. Indeed burnt patients with more TBSA, they have increased Neo level.

Table (3): The concentration of serum Neo (ng/ml) in studied groups according to TBSA %.

Studied groups TBSA%	No.	Mean concentration of serum Neopterin(ng/ml)	P value (probability) F-test
		Mean ± SE	
G1	18	6.74 ± 1.04 ab	0.003(HS)
G2	23	10.58 ± 2.71b	
G3	6	11.63 ± 2.88 b	
G4	3	36.73 ± 23.37c	
HC	17	15.51 ± 2.28 bd	
HS = Highly Significant (P<0.01); Different numbers mean significant			

As far as IL-1β, the estimated mean serum concentration in studied groups revealed highly significant differences (P<0.01) in mean serum concentration of burn patients

groups (G1),(G2),(G3),(G4) and HC (Table 4). Significant differences also revealed comparing G4 group versus all groups and HC. . Indeed burnt patients with more TBSA, they have increased IL-1β level.

Table (4): The descriptive statistics for the mean concentration of IL-1 β (pg/ml) in studied groups according to TBSA %.

Studied groups TBSA%	No.	Mean concentration of serum IL-1 β (pg/ml)	P value(probability) F-test
		Mean ± SE	
G1	18	0.22 ± 0.04a	0.001(HS)
G2	23	0.41± 0.14a	
G3	6	0.66 ± 0.31a	
G4	3	4.09 ± 3.34b	
HC	17	0.18 ± 0.01a	
HS = Highly Significant (P<0.01); Different numbers mean significant			

Concerning the estimation of mean serum concentrations of studied biomarkers in survivor and non-survivor burn patients, the mean serum level of survivor burn patients of Neo and IL-1β were increased non significantly in non-survivor burn patients, Table (5).

Table (5): Comparison of mean serum concentration of Neo and IL-1β in both survivor and non survivor burn patients.

Biomarkers	Survivor patients No=32	Non-Survivor patients No=18	P value(Probability) t-test
	Mean ± SE	Mean ± SE	
Neo (ng/ml)	7.34 ± 1.12	17.2 ± 4.95	0.068(NS)
IL-1β(pg/ml)	0.24 ± 0.04	1.43 ± 0.67	0.098(NS)
NS= Non-Significant (P>0.05).			

However, mean serum concentration of studied biomarker in survivor burn patients and healthy control, revealed highly significant difference (P<0.01) , (Table 6).

Table (6): Comparison of mean serum Neo and IL-1β in survivor burn patients and healthy control.

Biomarkers	Survivor patients No.32	Healthy Control No.20	P value(Probability) t-test
	Mean ± SE	Mean ± SE	
Neo (ng/ml)	7.34 ± 1.12	15.5 ± 2.288	0.004(HS)
IL-1β(pg/ml)	0.24 ± 0.04	0.188 ± 0.018	0.259(NS)
HS= Highly Significant (P<0.01), NS= Non-Significant (P>0.05).			

Regarding the estimation of mean serum concentration of studied biomarker in non-survivor burn patients and healthy control, Table (7) explains the distribution of different laboratory biomarkers between non-survivor burn patients and healthy control.

Table (7): Comparison of mean serum Neo and IL-1β in non- survivor burn patients and healthy control.

Biomarkers	Non-Survivor patients No.18	Healthy Control No.20	P value (Probability) t-test
	Mean ± SE	Mean ± SE	
Neo (ng/ml)	17.2 ± 4.95	15.5 ± 2.288	0.759(NS)
IL-1β(pg/ml)	1.43 ± 0.67	0.188 ± 0.018	0.083(S)
S= Significant (P<0.05) ; NS= Non-Significant (P>0.05).			

Table (8) demonstrate mean serum concentration of different laboratory biomarkers of (20) burn patients, there were statistically no significant difference (P>0.05) observed when compared the mean serum concentrations of IL-1β at admission and (10) day post burn (DPB). Highly significant difference (P<0.01) observed when compared mean serum concentration of Neo with mean serum concentration of Neo in (10) day post burn.

Table (8): Comparison of mean serum Neo and IL-1β in burn patients at admission and 10 days post burn.

Biomarkers	Burn patients at admission (No. 20)	Burn patients 10 DPB (No. 20)	P value(Probability) t-test
	Mean ± SE	Mean ± SE	
Neo (ng/ml)	10.04 ± 2.04	19.55 ± 2.78	0.009(HS)
IL-1β(pg/ml)	0.57 ± 0.35	0.3 ± 0.1	0.466(NS)
HS= Highly Significant (P<0.01),S= Significant (P<0.05) ,NS= Non-Significant (P>0.05),DPB: day post burn.			

Discussion

Regarding the body extended to burn insult, current study showed that mean of TBSA% burned were (35.03%) ranged (1-86%), in which the highest number 23 (46%) of burn victim fell in the 2nd group (G2) (26-50%), with mean age (22.49) years old and mean TBSA% burned (37.28%). Similar finding were reported by Othman and Kendrick[8]. Throughout this study, the mean TBSA in admitted burnt was (35.03%) ranged (6-100%), although the majority of burn patients 23 (46%) had TBSA burnt (37.28%) located in 2nd group, the mean TBSA burnt

reported from the countries of the East Mediterranean Region of the WHO program was variableand ranges from 10% to 48% [9].

In present study, Neo levels were found to be no significant difference in burn patient when compared with HC. This is agree with study of Jin *et al*, [10], however this is disagreement with study of Yao *et al.*, [11]. Following a severe burn injury, protein degradation and catabolism characteristic of hypermetabolism are exacerbated by the systemic inflammatory response resulting in compromised structure and function of muscle, skin, heart, liver, and immune system [12]. Mean serum concentration of Neon in studied groups, revealed no difference between groups versus HC as well as 4th group (G4). Meanwhile only significant elevation obtained if compared 1st group (G1) versus HC. The result of present study was similar to that reported by Mommsen *et al*., [13]. Monocytes /macrophages are good protectors of the body's, involved in host defense mechanisms by ingesting, killing and releasing a large number of mediators [14]. Endothelial damage and risk for septic complications cause increase concentrations of Neo, Indeed it is consider as a biochemical marker of cell-mediated immunity [15].

Present study referred to significant result when compared groups of different body surface area burned together, for instance 1st group (G1) versus 2nd (G2), 1st (G1) and 3rd group (G3) independently as well as 2nd group (G2) versus 3rd group (G3). The obtained results are in good agreement with other results[16]. Increased levels of endogenous interferon γ caused by Neo biosynthesis in inflammatory state, which was directly related to the extent of systemic T-lymphocyte activation. One of the most important events in the pathophysiology of sepsis appears to be massive and uncontrolled activation of macrophages and dendritic cell [11]. In this study, the Neo levels in patients were elevated higher significantly than control group. This may be because Neon is a cellular immunity biomarker, and increased their levels may reflect septic complications or depended on the severity of tissue damage and survival time.

Concerning the mean serum concentration of IL-1β in studied groups, current study revealed higher significant difference when mean concentration of IL-1 β of various sizes of TBSA% burned compared with HC subject. This finding was consistent with study reported by Hu et al., [17], who found that IL-1β concentration are influenced by multiple factors, such as age, burn size, time after burn injury and complications.

Actually, when comparison conducted independently between various groups of body surface burned with HC, statistical analysis revealed significant elevation of IL-1β concentration, G1, G2 and G3 while when compared with HC result was not significant, similar result obtained when comparison conducted between G1 versus G2, G1 versus G3 and G2 versus G3 whereas 1st group (G1) and 4th group (G4) ,G4 versus HC, G3 versus G4 and 2nd group (G2) and 4th group (G4) when compared with HC independently referred to highly significant differences ($P<0.01$).

Barber *et al*., (2008)[18]. Studied the relation between burn size (20%, 30%, 40%, and 60% TBSA) and cytokine concentrations (TNF α , IL-1 beta and IL-6) at one time point (24 hours) after burn injury, and observed burn size-dependent increases. IL-1β increased with the injury and decreased over time, this cytokine showed no significant difference between the burn size groups, but presented significant changes over time [19].

The finding in this study indicate that early after burn injury there is a correspondence of IL-1 beta with certain host

responses, but these correlations disappear with the progression of illness. In general, IL-1 beta appear to be poor indicators of prognosis during burn injury; however, the association of mortality with low circulating IL-1 beta values supports the concept of IL-1 beta as being an essential mediator of host defense[20].

Regarding the result which belong to survivor burn patients the mean serum concentration of Neo and IL-1β, no significant difference were found when compared to mean serum concentration of non-survivor burn patients. However, no significant differences observed in serum Neo concentration between patients who survived and those who did not survive. Similar finding have been demonstrated by Ahmad and Esraa, [21].

Stimulated T-lymphocytes produce a soluble factor (gamma interferon) that triggers Neo formation by monocytes/macrophages [10]. In the setting of acute thermal injury, stimulation of this T lymphocyte-macrophage axis could be affected by different antigenic stimuli, such as the bacterial endotoxin LPS, lipid protein complex or denatured collagen in the burn eschar[11]. There are two reasons for delayed increase Neo level might be due to the first is that the strongest inducer of Neo appears relatively late .The second is due to that the Neo production is related with production of reactive oxygen species by enthused immunocompetent cells. However, no significant differences observed in serum IL-1β concentration between patients who survived and those who did not survive. Similar finding have been demonstrated by [22]. Significant elevations of plasma IL-1β have been detected in healthy humans injected with LPS and in patients with septic shock and burns [20].

The present study were not compatible with other studies [20]. During early period after burn injury there is a relation between IL-1 beta and certain host responses, but these correlations disappear with the illness progression. In general, IL-1 beta act as poor indicators of prognosis during burn injury; however, IL-1 beta being an essential mediator of host defenses through the association of mortality with low circulating IL-1 beta values. There are significant time-dependent changes in plasma concentrations of IL-1beta after serious burns. IL-1beta has a role in the host's response to infection; and it may influence outcome. The inflammatory response starts immediately after burn trauma and persists for almost 5 weeks post burn [23].

As far as the results of present study there were no significant differences observed of mean serum concentration of IL-1β between survivor burn patients (0.02) and HC (0.188). On the other hand highly significant differences observed when mean serum concentration of Neo of survivor patients (7.34) when compared with mean serum concentration of HC(15.5) respectively. The obtained results showed concur with results of preceding publishers reported by Abdel-Hafez et al., [24].

As shown in present study the significant elevation of the biomarkers Neo and IL-1β in deceased burn patients if compared with HC, might possibly due to installation of post burn complication that can lead to death, in addition the total 18 (36%) deceased patients in this study have been burnt with TBSA (69.70%) . This is consistent with Purcaru and Bogdan, [25]. who stated that burn size more than 25% TBSA exposed the burn patient vulnerable to systemic manifestation such as hypovolaemic shock and acute phase systemic inflammation.

Explanation of the discrepancy of these biomarkers mainly result from the catastrophic burn insult, which possibly disturbing almost all essential organ including skin, immune system, heart, liver, lung, kidney and brain[26]. Most fatalities

(65%) seem to be linked to burn-induced MOF and 93% of burn patients present with clinical signs of the systemic inflammatory response syndrom before succumbing to their injuries [27]. Levels of Neo and IL-1β increased in burnt patients with increased TBSA%, non-survivor compared with survivor patients and HC. Indeed levels of Neo level increased in (10) day post burn.

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