ORIGINAL ARTICLE

A Study of Serum C3 and C4 Levels in Burn Patients: Possible Prognostic Markers?

Sanjay Sadhu, Kiran Bhat[1]

Abstract

Background: Burns are a public health problem across the world and a major burden on health care resources. Identifying major burns including those more likely to develop sepsis will help a lot in their management. ***Purpose:*** This study was undertaken to assess the changes in complement levels in burns and their possibility of acting as a marker for severe and complicated burns. ***Material and Methods:*** This was a prospective case control study. Sixty burn patients were enrolled in the study. Their serum C3 and C4 levels were assessed at the time of presentation and at 10 days later. Fifty healthy subjects were enrolled as controls and their C3 and C4 levels were assessed. ***Results:*** The age group of patients was 3 to 63 years. There were 47 male patients and 13 female patients. There was a significant decrease in mean post treatment levels of serum C3 (141.82 ± 78.07 mg/dl) and C4 (25.09 ± 11.22 mg/dl) as compared to pre-treatment levels of C3 (188.22 ± 92.35 mg/dl) and C4 (46.49 ± 15.22 mg/dl) in the burn patients. The fall was more in patients with major burns. ***Conclusion:*** There are changes in serum complement component levels in burn patients which have the potential of being prognostic markers. The role of complement system on the overall pathophysiology of burn patients also needs to be studied more extensively.

Key Words

Complement system, Burns, C3, C4

Introduction

Burn injuries are one of the major public health concerns worldwide, especially in the developing countries. It is estimated that annually 200,000 people die due to burn injuries around the world (1). Among these 30-50% of the deaths are attributed to sepsis and its sequelae (2). Out of total deaths, 57% occur in South East Asian countries. In India the incidence of burn cases is 6-7 million per year (3).

Major trauma including burns induce inflammatory response in the body. Complement system is activated as a part of the inflammatory response which leads to release of complement components and their utilisation (4). Complement components especially C3, C4 and C5 therefore get depleted. With improvement in patient's condition the complement levels are restored towards normal. However, in severe burns the complement system remains activated and the complement levels remain persistently low. These patients subsequently are more prone to complications including sepsis and death. Thus, persistently low levels of complement indicate high chances of adverse outcome of the patient.

Major burns have a prolonged course with many patients succumbing to the burns. One of the major causes

Department of Plastic Surgery, Shri Guru Ram Rai Institute of Medical and Health Sciences, and [1]Department of Biochemistry, Himalayan Institute of Medical Sciences, Jolly Grant - Dehradun, Uttarakhand, India

Correspondence to: Dr. Kiran Bhat, Associate Professor, Department of Biochemistry, Himalayan Institute of Medical Sciences, Jolly Grant, Dehradun, Uttarakhand, India

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of mortality in burns is septicaemia. The pathophysiology of sepsis is characterised by impairment of various body systems at cellular, tissue specific and functional levels (5). Early diagnosis of sepsis and institution of appropriate therapy can avoid mortality in many of these patients. It has been shown that in patients with septicaemia, delay of few hours in diagnosis and starting of appropriate therapy reduces the chances of survival significantly (6). In present study we evaluated serum C3 and C4 levels in burn patients and assessed their relation to development of sepsis, patient outcome and possible use as prognostic marker.

Material and Methods

All burn patients who were admitted to our hospital and did not fit into exclusion criteria were included in the study. The exclusion criteria were:

1. Patients with systemic illnesses like diabetes, systemic lupus erythematosus, rheumatoid arthritis and tuberculosis.
2. Patients already having local/systemic infection prior to burn e.g., endocarditis, post streptococcal glomerulonephritis, septicaemia.
3. Patients having malignancy.

All burn patients other than those with exclusion criteria mentioned above irrespective of mode/type of burn admitted to Shri Mahant Indiresh Hospital between Jan 2018 and June 2019 were included in the study. Blood sample of 3ml was collected in yellow top gel separated vacutainer twice from each patient, once at the time of admission and then ten days after burn injury. Serum C3 and C4 levels were estimated in each sample. Fifty healthy subjects who were employees/students of the institute were included as controls in this study. Only one sample was collected from controls. Cases and controls were matched with respect to age and sex to minimise bias.

Principle: C3 and C4 were estimated using Ortho Clinical Diagnostic 5600 auto analyser based on immunometric immunoassay. Normal range for serum C3 is 90-180mg/dl and for serum C4 is 20-50 mg/dl using this method.

This was a prospective case control study approved by Institutional Ethics Committee vide letter number SGRR/IEC-62/18. The analyses were done at the Department of Biochemistry, Central Laboratory, Shri Mahant Indiresh Hospital, Dehradun. Consent was taken from cases and controls for participation in the study.

Detailed history was obtained and physical examination done at the time of admission. Essential parameters like pulse rate, blood pressure, respiratory rate and urine output were monitored throughout. Extent/percentage of burns was calculated using Wallace's rule of nine and treatment started. Appropriate investigations such as complete blood count, blood sugar, renal function tests, serum electrolytes, blood culture were done as per protocol and requirement.

Results

A total of 60 burn patients were studied (*Table 1*). The age of burn patients varied from 3 to 63 years, mean age being 24.55 ± 17.06 years. There was a preponderance of male patients in our study with 46 (76%) of patients being male. Out of total patients 31 (51.77%) had thermal burns, 25 (41.7%) had electric burns and 4 (6.7%) had chemical burns. For the purpose of analysis, the patients were stratified into 2 main groups:

1. Mild to moderate burns where ≤ 20% of the total body surface area was burnt.
2. Severe burns where >20% of total body surface area was burnt.

The number of patients having mild to moderate burns was 33 (55%) and 27 (45%) had severe burns.

The mean pre-treatment serum C3 level was 188.22 ± 92.35 mg/dl (range 54.95 – 580.29 mg/dl) (*Table 2*). Mean C3 levels after 10 days were 141.82 ± 78.07 mg/dl (range 53.0 – 406.0 mg/dl). The mean pre-treatment

Table 1: Baseline Characteristics of the Study Subjects

Characteristic		No.	%
Age Group	0 – 18yrs	23	38.3
	19 – 40yrs	25	41.7
	41 – 60yrs	10	16.7
	>60yrs	2	3.3
Sex	Male	46	76.7
	Female	14	23.3
Type of Burn	Thermal	31	51.7
	Chemical	4	6.7
	Electrical	25	41.7
Percentage of Burn	≤20%	33	55.0
	>20%	27	45.0

Table 2: Baseline Mean Parameters in Study Subjects

Characteristics	Mean	SD	Range
Age	24.55	17.06	2 – 65
Pre-treatment C3	188.22	92.35	54.95 – 580.29
Post-treatment C3	141.82	78.07	53.0 – 406.0
Pre-treatment C4	46.49	15.22	17.0 – 76.0
Post-treatment C4	25.09	11.22	10.0 – 62.0

serum C4 level was 46.49 ± 15.22 (range 17-76 mg/dl) and after 10 days mean serum C4 level was 25.09 ± 11.22 mg/dl (range 10 – 62 mg/dl). The post treatment levels of C3 and C4 show a significant decrease compared to pre-treatment levels (*Table 3*).

Table 3: Comparison of Pre and Post Treatment Mean of C3 and C4

Variable	Pre-treatment		Post-treatment		*p* value*
	Mean	SD	Mean	SD	
C3	188.22	92.35	141.82	78.07	0.001
C4	46.49	15.22	25.09	11.22	0.001

* Paired-Samples T Test

Table 4: Comparison of Mean Value of C3 and C4 with Degree of Burn

Variable	≤ 20% Burn		>20% Burn		*p**
	Mean	SD	Mean	SD	
Pre-treatment C3	181.93	91.32	195.89	94.76	0.57
Post-treatment C3	155.03	87.88	125.68	61.90	0.15
Pre-treatment C4	45.01	13.99	48.31	16.69	0.41
Post-treatment C4	27.43	13.21	22.22	7.43	0.07

*Independent-Samples T Test

The results of the two sub-groups of burn patients are shown in *Table 4*. The decrease in serum levels of C3 and C4 after 10 days is more in patients with major burns than in patients with mild/moderate burns. However, the difference is not statistically significant when the two groups are compared with each other. The serum C3 and C4 levels in second sample at 10 days were markedly lower in patients who developed septicaemia and/or expired. The mean duration of hospital stay was lesser in first group as compared to those in second group.

The main causes of death were septicaemia and cardiac involvement (especially in electric burn patients). The most common organism isolated in wound swab culture was Pseudomonas aeruginosa.

Discussion

Activation of complement system is part of body response to tissue injury, including burn injury, and plays an effective role as an innate response system. Numerous studies have been done to study the response of complement system to injury and the effect of complement system locally as well as systemically.

The blood levels of C3, C3a and C5a are increased following burns which may remain elevated for months after the burn injury. However, prior to this long-term increase, C3 and C3a levels have been shown to be lowered for first few days after the burn (7). This has been ascribed to capillary leakage and consumption opsinopathy. Modi *et al.* (8) in their study showed that the C3 levels remained persistently decreased in patients with severe burns who did not survive or who developed sepsis. It was suggested that persistently low levels of C3 could be used as predictor for infections, septicaemia and mortality in burn patients. The result of our study correlates with this. In our study we observed a significant fall in serum C3 and C4 levels after 10 days. The initial levels were comparable to the levels in control group.

Despite all the progress that has been made in the management of burns, the prognosis remains poor for many of these patients, especially, those with major burns. Higher the percentage of body surface area burnt; graver is the outcome. The major causes of mortality in burn patients are septicaemia, pulmonary complications and multiple organ failure. It would be helpful if the patients progressing towards these complications could be identified earlier as that would lead to early initiation of appropriate therapy and mortality could be avoided in at least some of these patients. As discussed above persistently low levels of complement could be markers for patients who are likely to have complicated course and poor outcome. Monitoring of complement levels could therefore act as guide for early institution of therapeutic measures and better salvage of these patients.

The various effects of complement in burns, both locally and systemically, have been the topic of many studies. The complement components have haemostatic, antibacterial and pro-inflammatory effects which enhance wound healing (9). Complement has also been shown to aid the clearance of debris from the wound. This inflammatory phase normally settles after a few days of trauma. However, in burn wounds, the inflammatory response can get prolonged which results in detrimental effects including endothelial damage, delayed wound healing, excessive wound contraction and hypertrophic scarring (9). The endothelial damage leads to increased vascular permeability and thrombosis (10). Inhibition of complement has been shown to reduce the detrimental effects of prolonged inflammation (11).

The complement system has been shown to adversely affect heart. It is also thought to adversely affect the liver, kidneys, brain, gastro-intestinal tract and skeletal muscle (4). Immunological approaches to therapy targeting the complement system have been suggested for improved outcomes (12).

Conclusion

The complement components C3 and C4 show a significant fall over time in burn patients. The fall is more marked in more severe and/or complicated burns. These patients are more likely to have an adverse outcome. A prolonged hospital stay is also likely in patients having marked fall in complement levels. Further studies can be directed towards the effect of complement in burn patients.

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Conflicts of Interest

There are no conflicts of interest.

References

1. Mathers C, Boerma T, Fat DM. The global burden of disease: 2004 update. *Geneva: World Health Organization*; 2008.
2. Williams FN, Herndon DN, Hawkins HK, Lee JO, Cox RA, Kulp GA, *et al.* The leading causes of death after burn injury in a single pediatric burn centre. *Crit Care* 2009;13(6):R183.
3. Gupta JL, Makhija LK, Bajaj SP. National programme for prevention of burn injuries. *Indian J Plast Surg* 2010;43(Suppl):S6-S10.
4. Korkmaz HI, Krijnen PAJ, Ulrich MMW, de Jong E, van Zuijlen PPM, Niessen HWM. The role of complement in the acute phase response after burns. *Burns* 2017;43(7):1390-99.
5. Hietbrink F, Koenderman L, Rijkers G, Leenen L. Trauma: the role of the innate immune system. *World J Emerg Surg* 2006;1:15
6. Kumar A, Ellis P, Arabi Y, Roberts D, Light B, Parrillo JE, *et al*. Initiation of inappropriate antimicrobial therapy results in a fivefold reduction of survival in human septic shock. *Chest* 2009;136(5):1237-48.
7. Jeschke MG, Gauglitz GG, Kulp GA, Finnerty CC, Williams FN, Kraft R, *et al.* Long-term persistence of the pathophysiologic response to severe burn injury. *PLoS One* 2011;6(7):e21245.
8. Modi S, Rashid M, Malik A, Shahid M. Study of complement activation, C3 and interleukin-6 levels in burn patients and their role as prognostic markers. *Indian J Med Microbiol* 2014;32:137-42.
9. Sinno H, Malhotra M, Lutfy J, Jardin B, Winocour S, Brimo F, *et al.* Accelerated wound healing with topical application of complement C5. *Plast Reconstr Surg* 2012;130(3):523-29.
10. van de Goot F, Krijnen PA, Begieneman MP, Ulrich MM, Middelkoop E, Niessen HW. Acute inflammation is persistent locally in burn wounds: a pivotal role for complement and C-reactive protein. *J Burn Care Res* 2009;30(2):274-80.
11. Cazander G, Jukema GN, Nibbering PH. Complement activation and inhibition in wound healing. *Clin Dev Immunol* 2012;2012:534291.
12. Boldeanu L, Boldeanu MV, Bogdan M, Meca AD, Coman CG, Buca BR, *et al.* Immunological approaches and therapy in burns (Review). *Exp Ther Med* 2020;20(3):2361-67.