Oxidative stress in burnt children

Jutkiewicz-Sypniewska J[1], Zembroń-Łacny A[2], Puchała J[3], Szyszka K[2], Gajewski P[1,2]*

[1] The Pediatric Surgical Ward, Public County Hospital in Gorzów Wielkopolski, Poland
[2] Institute of Physical Education in Gorzów Wielkopolski, Poland
[3] The Plastic, Reconstructive and Burn Surgery Department, University Children's Hospital of Cracow, Poland

Abstract

Background: One of the effects of burn injury is production of reactive oxygen species increasing general-structural damage. Such a condition is called oxidative stress. The purpose of this research was to find out whether oxidative stress is present in burnt children treated routinely and, if so, in which phase of the disease it is the most severe and how long it persists.

Material and methods: The study was carried out on a group of 84 burnt children. The patients were divided into 2 groups: lightly burnt (LB-N:55) and moderately to severely burnt (SB-N:29). Blood samples were collected based on hospitalization period within the 1st, 2nd, 3rd, 7th and 21st day, respectively, following the injury. Total antioxidative capacity (TAC) in plasma and concentration of lipid peroxidation products (TBARS) in red blood cells were estimated. The test results were compared to control group of 40 healthy children.

Results: The research showed a statistically significant decrease in TAC in both groups of burnt children. The TBARS concentration was increased in both groups within the 1st day following burn injury and maintained the high level throughout the research continuation. No statistically significant differences between LB and SB groups were recognized.

Conclusions: The observed changes in the tested parameters are attributable to oxidative stress occurring in burn disease. For this reason, burn – injured children are recommended to receive exogenous antioxidants.

Key words: children, burns, oxidative stress, lipid peroxidation, antioxidants.

* CORRESPONDING AUTHOR:
Faculty of Physical Culture, Department of Medical Sciences
ul. Estkowskiego 13, 66-400 Gorzów Wlkp., Poland
Tel: +48 95 7279222
e-mail: agzem@gorzow.home.pl (Agnieszka Zembroń-Łacny)

Received 01.08.2005 Accepted 24.01.2006

Introduction

Among various pathophysiological mechanisms in burn disease, the subject of reactive oxygen species (ROS) has been drawing interest since the early 70's. The ROS are mostly free radicals including such molecules as: superoxide radical anion, hydroperoxyl radical, singlet oxygen and hydrogen peroxide. The results of previous research show that burn injury is followed by ROS generation due to hypoxia – reperfusion (dehydrogenase change into xanthine oxydase in vascular endothelium cells) and activation of immune system (NADPH oxydase reaction in phagocytic cells). The excessive increase in ROS level can lead to oxidative stress defined as prooxidant – antioxidant equilibrium disturbance [1,2].

The most frequently used marker of oxidative stress is a measurement of concentration of lipid peroxidation products including lipid peroxides, aldehydes (malonyldialdehyde MDA), alkenales. The lipid peroxidation results from ROS attacking mainly the cell membranes, which leads to an attenuation of membrane flexibility, disturbance of asymetric distribution of membrane phospholipides, elevation of permeability for hydrogen ions, dysfunction of Na^+K^+ and $Ca^{2+}Mg^{2+}$ ATPases, etc. [3,4].

Due to adverse events of ROS generation, cells are equipped with protective mechanisms against oxidative stress. It is an antioxidative defence involving low-molecular compounds (α-tocopherol, ascorbic acid, β-carotene, glutathione, Q-coenzyme, uric acid, bilirubin, etc.), enzymatic compounds (superoxide dismutase SOD, catalase CAT, glutathione peroxidase GPx, etc.), proteins transporting and storing ions of metals (ferritin, transferrin, ceruloplasmin, metalothionein). Most of the mentioned antioxidants affecting total antioxidant capacity (TAC) are found in plasma. The TAC means an ability to scavenge ROS and prevent lipid peroxidation. The marker was introduced by Ingold and Burtin as an antioxidative potential in subjects. The TAC involves mainly includes uric acid, ascorbic acid, α-tocopherol and albumin [5,6].

The world literature of medicine provides with little infor-

mation on the role reactive oxygen species play in burn pathogenesis in humans, particularly, children. Attempts to diminish burn effects, namely, oxidative stress by antioxidants including α-tocopherol, superoxide dismutase have been made, however, without consistent results [7-10].

The purpose of the research was to find out:

– whether oxidative stress occurs in burnt children treated according to methods of burn treatment,

– in which phase of the disease the severity and persistence of oxidative stress is the greater

– whether treatment requires adjusting intended to prevent or attenuate harmful effects of reactive oxygen species reaction on a patients.

Material and methods

Subjects

The research test was carried out on a group of 84 burnt children admitted to the pediatric surgery ward in County Hospital in Gorzów Wlkp. from 1998 to 2000. The children's age ranged from 5 months to 14 years old. The patients were divided into 2 groups:

– LB – lightly burnt – first and second – degree burns, less than 10 percent of the total body area (TBA) (55 children)

– SB – moderately to severely burnt (second – degree burn, more than 10 percent of the TBA to third – degree burn requiring over 7 days of hospitalization (29 children).

Average duration of hospitalization for slightly – burnt children and moderately to severely burnt ones was 3.5 days and 16.5 days, respectively.

The test covered only those children who were admitted to hospital within the first day following burn injury. The patients were treated routinely by methods accepted in the general and local burn treatment of children. Fluid resuscitation was carried with the Parkland method. It was being adjusted based on clinical evaluation of the patient's general condition, aiming at reaching minimal diuresis 1 ml/kg per hour. The intravenous co-administration of calcium preparations and vitamin C was initiated within the first day following burn injury. In addition, daily treatment with 20% albumin solution at 2-3 g/kg b.m. was implemented in the patients within the 36th-48th hour. Morphine i.v. and paracetamol per rectum were applied as analgesics. Based on a degree of burn injury antibiotic therapy was begun either within the first day or later adequately to an antibiogram received from the burn wound swab. The children were fed orally or through the nasogastric tube soon after they had recovered from the trauma. Parenteral nutrition following the procedure by Sporadyk and Puchała was applied [11,12]. In the burn injuries requiring surgical treatment an early necrectomy was performed within the 3rd to 5th day after burn injury usually simultaneously with the skin grafting. In the burn injuries of varied severity or particular anatomic location (fingers and intradigital spaces) dermabrasion was applied. In total, there were 27 such treatments performed in 16 patients. None of them required escharotomy.

Vein blood samples were collected from both groups on the 1st and the 3rd day after burn injury and, additionally, from the group of moderately to severely burnt on the 7th, 14th and 21st day, responsively, appropriate for duration of hospitalization. The blood samples were always collected together with samples for routine laboratory tests. The test results in the injured children were compared with the control group of 40 healthy children admitted to hospital for planned surgical treatment.

The assembly has got an approval of Bioethical Committee at District Chamber of Physicians in Gorzów Wlkp. to carry out the research. In case of each burn-injured child and the child from control group a written permission was given by their parents to use the blood samples in order to estimate oxidative stress markers.

Biochemical analysis

The vein blood samples for estimation of oxidative stress parameters were collected into polyethylene tubes containing potassium EDTA. The blood was centrifuged (750 g, 4°C, 10 min), plasma was removed and stored at -20°C until analysis. Erythrocyte fractions were resuspended and washed three times with cold isotonic saline solution. Washed erythrocytes were stored at -20°C until analysis (up to seven days).

In the erythrocytes lipid peroxidation was estimated using the measurement of thiobarbituric acid – reactive substance (TBARS) level according to Buege and Aust method [13].

In the plasma total antioxidant capacity (TAC) was determined using a diagnostic kit Randox (UK).

Hemoglobin was estimated with Drabkin standard method.

Statistical analysis

All data are presented as mean ± SD and analyzed by "Statistica for Windows" program, using U Mann-Whitney test and a model of multidimensional regression. The accepted level of significance was $p<0.05$.

Results

The obtained test results are presented in *Tab. 1,2* and *Fig. 1,2*.

The concentration of lipid peroxidation products (TBARS) was statistically significantly higher in the groups of lightly burnt children (LB) and moderately to severely burnt (SB) in comparison with the control group. The highest average values of TBARS concentration were observed in SB group within the 21st day of the examination. Analyzing the diagram of distribution of the TBARS concentration values according to the test duration (*Fig. 1*), a constant and uniform increase in TBARS concentration was observed in both groups of burnt children throughout the whole period of the examination. No statistically significant changes between LB and SB groups were observed.

Total antioxidant capacity (TAC) was significantly lower in groups of lightly burnt (LB) and moderately to severely burnt (SB) children in comparison with the control group. The lowest average TAC values were observed in LB and SB groups within 3rd and 7th day, respectively, following burn injury. Distribution of TAC concentration according to test duration is illustrated in the *Fig. 2*. It indicates that the concentration of antioxidants was lower in LB group compared to control group on the

Table 1. **Concentration of lipid peroxidation products (TBARS) in subjected groups (mean±SD)**

	Control	LB-1	LB-3	SB-1	SB-3	SB-7	SB-14	SB-21
N	40	55	23	29	29	29	13	7
TBARS μmol/gHb	3.03±0.65	5.66±1.56*	5.33±1.35*	5.67±1.75*	5.63±1.58*	5.88±1.28*	5.98±0.88*	6.57±1.25*

Table 2. **Total antioxidant capacity (TAC) in subjected groups (mean±SD)**

	Control	LO-1	LO-3	CO-1	CO-3	CO-7	CO-14	CO-21
N	40	55	23	29	29	29	13	7
TAS mmol/l	1.34±0.20	1.28±0.28	1.05±0.20*	1.19±0.20*	1.11±0.16*	1.06±0.17*	1.18±0.22*	1.13±0.26*

* Statistically significant differences ($p<0.05$) in relation to control group

Figure 1. **Distribution of lipid peroxidation products (TBARS) in accordance with examination time**

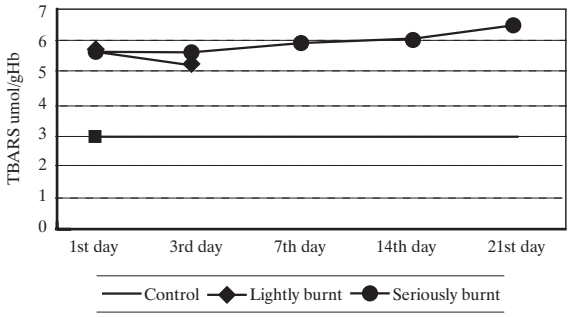
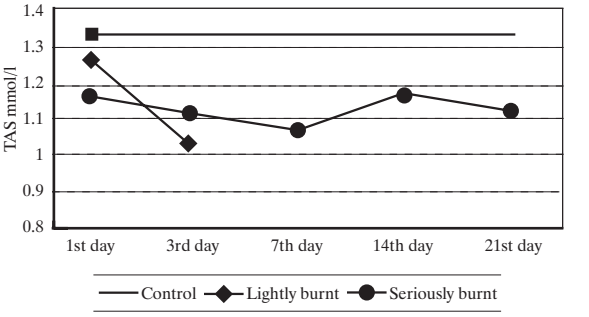


Figure 2. **Distribution of total antioxidant capacity (TAC) in accordance with examination time**



1st day following injury ($p>0.05$) and on the 3rd day of the clinical trial there was a further decrease in TAC, which was significant statistically comparing with control group. In SB group a statistically significant decrease of the TAC was observed from the very 1st day ($p<0.05$), which maintained until the research was completed. No statistically significant differences between LB and SB groups were noted on particular days of the research.

Discussion

A thermal injury is followed by a significant increase in generation of ROS in the body. Within the early phase of the disease ROS generation occurs in hypoxia and damaged capillaries in the injured area. Such a condition causes the xanthine dehydrogenase to convert into xanthine oxydase using molecular oxygen as an electron acceptor after reperfusion. Its result is formation of superoxide radical anion [14,15]. In further phases of the disease ROS level may be similar to the initial one, but their source is different. Injury-activated neutrophils migrate not only to the injured skin area but also to remote organs, especially lungs. The activated neutrophils are considered to be the main source of ROS generation in further phases of burn disease [7,16]. Another source of ROS is the release of ferric and copper ions from the damaged cells inducing an increase in hydroxyl radical production by Fenton reaction. The role of the released ferric and copper ions in ROS generation is confirmed by tests proving that ferric chelator (deferoxamine) administration in the post-burn resuscitation decreased significantly a demand for fluids and diminished the red cells hemolysis [17,18].

Analyzing the burn injury influence on concentration of lipid peroxidation products (TBARS) in red cells in burnt children, their statistically significant elevated level was recognized in all the tested groups. An increase in TBARS concentration appeared as early as on the 1st day following burn injury. The finding is consistent with most observations apart from Guerbez [19] who did not confirm any increase in products reacting with thiobarbituric acid in lung tissue, liver or stomach of burnt rats. Neither did the author recognize any increase in protein peroxidation level. In other studies there was observed enhanced peroxidation of lipids in burnt skin and lungs, with their highest values appearing 15 min and 2 h after burn injury and, respectively, after 30 min and 30 h, in plasma [20]. Besides, the observations confirmed a markedly elevated level of lipid peroxides in the skin and plasma 1 h after burn injury, reaching 6-times higher level 3 h following the injury compared to control group [21]. Other tests showed an increased level of lipid peroxidation products in the plasma and in lungs and liver within the 4th day after burn [22,23]. An increase in conjugated dienes, lipid peroxides and malonyldialdehyde contents in plasma and lung tissue was observed in animals 2 h after burn injury [24]. In an experimentally produced multiorgan failure syndrome (MOF) in rats, there was recognized an increase in TBARS within the 1st and 2nd day [25]. The findings are in agreement with clinical test in burnt patients, expressing an increase in lipid peroxidation products concentration as early as within the 8th h, the 1st and 2nd day after burn injury [26-28].

Lipid peroxidation was strongly expressed, which was confirmed by the persistence of elevated TBARS concentration in the burn disease (in the present studies – till the 21st day after burn). The results obtained by other authors were consistent. However, it should be noted that most of the experimental tests were carried out within short time periods. Szpringer et al. [24] were observing an increase in plasma lipid peroxidation products concentration within the 48 h, Dargani et al. [29] – in the lungs and liver within the 3rd day, Demling and Lalonde [30] in the plasma within the 5th day after burn injury. Nishigaki et al. [21] recognized an elevated level of lipid peroxidation products in the burnt skin and plasma maintaining till the 7th day, since which it returned to control group values (the tests were carried out till the 28th day). Van Bebber et al. [31] doing experimental tests on MOF observed an increased level of the TBARS until the 14th day. The results of clinical observations show high concentration of lipid peroxidation products maintaining until completion of the tests: Hongming et al. [26] – till the 3rd day, Wooliscroft et al. [27] – till the 5th day and Kumar et al. [28] – till the 10th day, respectively, after the injury.

The increase in concentration of TBARS is likely to be related to the specificity of injury, not its severity. It is confirmed by the lack of statistically significant differences in TBARS concentrations between LB (first- and second-degree burn) and SB (second- to third-degree burns) groups. Besides, the fact is stated by other authors who observed no correlation between malonyldialdehyde concentration and the area of burn [28]. Moreover, the levels of lipid peroxidation products were comparable in the patients with either severe or light burn [9]. In the tests by Gosling et al. [32] concentration of lipid peroxidation products was higher in more severely burnt patients, but had no correlation with the patients' death rate though. Madsuda et al. [33] found out that changes in antioxidants concentration and the level of oxidative damage in the plasma appear only with deeper skin layer injury.

There are much fewer studies concerning evaluation of total antioxidant capacity (TAC) of plasma than those describing changes in concentration of lipid peroxidation products. Our results show that TAC level was decreased in both groups of burnt children. In the group of moderately to severely burnt, total antioxidant status of the plasma did not return to control values until the 21st day following burn. Demling et al. [34] presented their test results showing that the decrease in antioxidants concentration and the increase in lipid peroxidation with inhale burn injury. Cetinkale et al. [22] observed TAC decrease 24 h after burn injury. The analysis of particular elements of total antioxidant status in burn blisters suggested that protein concentration was by 54% lower in bluster fluids than in plasma and concentration of bilirubin by 32% respectively. However, uric acid level was unchanged in plasma [35]. Other clinical tests showed a significant decrease in plasma α-tocopherol, thiols and increase in conjugated dienes levels in burnt patients [36].

Few studies confirmed the correlation of oxidative stress indicators with the patients' clinical condition and the burn disease course [32-34]. However, in our tests no correlation between the children's clinical condition and the changes in lipid peroxidation products concentration was recognized. The TBARS and TAC concentrations were the same both in the group of lightly burnt and moderately to severely burnt children. The lightly burnt children were discharged from hospital in good clinical condition after 3.5 days of hospitalization. In LB group the levels of the tested parameters on the 3rd day were not statistically different from the levels obtained in SB group hospitalized for 16.9 days or the patients requiring surgical treatment. It is worth noticing that values of TBARS and TAS concentrations at the beginning of the burn disease did not differ from the results obtained on the 21st day in the severely burnt children during recovery phase.

Total antioxidant status (TAC) in the plasma involving the activity of all the compounds being able to play a role of free radicals scavengers (uric acid, vitamin C, E, bilirubin, albumin) appears to be a very sensitive marker of the oxidative stress. The decreased TAC together with an elevated level of lipid peroxidation products persistent through the burn disease duration is attributable to ROS generation and oxidative stress. Immunological defence mechanisms in burnt children are disturbed not only due to the damaged skin – an important protective barrier, but also repeated surgical treatments under general anaesthesia, antibiotics, ointments and other therapy applied locally on the burn wound. Besides, the increased catabolism, a nitric imbalance and other metabolic irregularities make the contribution to oxidative stress. The described changes in the oxidative stress markers are likely to play a role in the pathophysiology of immunosuppression present in burnt patients and response of the body to so-called "second hit" (f.e. sepsis) affecting the development of MODS [37,38].

Conclusions

Burn injury induced oxidative stress in the examined groups expressed as an increase in concentration of lipid peroxidation products (TBARS) in red blood cells and a decrease in total antioxidant capacity (TAC) in plasma. The process started immediately after the injury and persisted through the whole course of study (till the 21st day after burn).

The observed irregularities were caused by the specificity of injury regardless of its severity and the described changes were comparable in both groups of burnt children.

An attenuation of total antioxidant capacity (TAC) in plasma and enhancement of lipid peroxidation (TBARS) in erythrocytes maintaining during the study may be related to immunosuppression present in burnt children and their high sensitivity to so-called "second hit". For the reason, burn-injured children are recommended to continue long-term supportive therapy wit exogenous antioxidants.

References

1. Andrzejak R, Goch JH, Jurga M. Wolne rodniki i ich znaczenie w medycynie. Post Hig Med Dośw, 1995; 49: 531-49.
2. Bartosz G. Druga twarz tlenu. Warszawa: PWN; 1995.
3. Halliwell B, Gutteridge JMC. Lipid peroxidation, oxygen radicals, cell damage, and antioxidant therapy. Lancet, 1984; 23: 1396-7.
4. Slater TF. Free-radical mechanisms in tissue injury. Biochem J 1984; 222: 1-15.
5. Stocker R, Frei B. Endogenous antioxidant defences in human

blood plasma. In: Oxidative stress. Oxidants and antioxidants. Sies H editor, London: Academic Press; 1991.

6. Uotila JT, Kirkkola AL, Rovarius M, Tuimala R, Metsa-Ketela T. The total peroxyl radical – trapping ability of plasma and cerebrospinal fluid in normal and preeclamptic parturients. Free Rad Biol Med, 1994; 16: 581-90.

7. Demling RH, La Londe C. Early postburn lipid peroxidation: Effect of ibuprofen and allopurinol. Surgery, 1990; 107: 85-93.

8. Lozano T, Guemes F, Santos FX, Obispo JM. Effect of superoxide dismutase on haematologic parameters and urinary changes after burn injury in rats. Eur J Plast Surg, 1993; 16: 263-6.

9. Thomson PD, Till GO, Wooliscroft JO, Smith DJ, Prasad JK. Superoxide dismutase prevents lipid peroxidation in burned patients. Burns, 1990; 16: 406-13.

10. Wu KY. Effect of superoxide dismutase and allopurinol on microcirculatory disturbances during burn shock. Zhonghua Zheng Xing Shao Shang Wai Ke Za Zhi, 1989; 319: 284-6.

11. Spodaryk M, Puchała J. Early total parental nutrition employing TPN v. 1.2.PATI Software in the treatment of children with massive burns. Surg Childh Intern, 1997; 5: 96-8.

12. Spodaryk M, Puchała J, Grochowski J. Nutritional support in combined treatment of severe burns in children – problems and effects. The International Symposium and Course on Burns and Fire Disaster, 3rd Jerusalem Meeting of MBC; 2000 Feb 13-16; Jerusalem, Israel.

13. Buege J, Aust S. The thiobarbituric acid assay. In: Techniques in free radical research. Rice-Evans CA, Diplock AT, Symons MCR editors. Elsevier Amsterdam London New York Tokyo; 1991.

14. De Bono DP. Free radicals and antioxidants in vascular biology: the roles of reaction kinetics, environment and substrate turnover. Quart J Med, 1994; 87: 445-53.

15. Kehrer JP, Jones DP, Lemaster JJ, Farber JL, Jaeschken H. Mechanisms of hypoxic cell injury. Toxicol Appl Pharmacol, 1990; 106: 165-78.

16. Nuytinck H, Xavier J, Offerman W. Whole body inflammation in trauma patients. Arch Surg, 1988; 123: 1519-23.

17. Demling RH, Lalonde C, Knox J, Youn Y, Zhu D, Daryani R. Fluid resuscitation with deferoxamine prevents systemic burn-induced oxidant injury. J Trauma, 1990; 31: 538-44.

18. Halliwell B. Oxidants and humans disease: some new concepts. FASEB J, 1987; 1: 358-64.

19. Guerbuez V, Corak DB, Yegen BC, Kurtel H, Alican I. Oxidative organ damage in a rat model of thermal injury: the effect of cyclosporin A. Burns, 1997; 231: 37-42.

20. Till GO, Hatherill JR, Tourtellotte WW, Lutz MJ, Ward P. Lipid peroxidation and acute lung injury after thermal trauma to skin. Evidence of role for hydroxyl radical. AJP, 1985; 119: 376-84.

21. Nishigaki I, Hagihara M, Hiramatsu M, Izawa Y, Yagi K. Effect of thermal injury on lipid peroxide levels of rat. Biochem Med, 1980; 24: 185-9.

22. Cetinkale O, Belce A, Konukoglu D, Senyuva C, Gumustas MK, Tas T. Evaluation of lipid peroxidation and total antioxidant status in plasma of rats following thermal injury. Burns, 1997; 23: 114-6.

23. La Londe C, Knox BSJ, Daryani R, Zhu D, Demling RH, Neumann M. Topical flurbiprofen decreases burn wound-induced hypermetabolism and systemic lipid peroxidation. Surgery, 1991; 109: 645-51.

24. Szpringer E, Marciniak A, Górny D, Bełtowski J. Dynamika peroksydacji lipidów w osoczu i płucach ciężko oparzonych szczurów. Roczniki Oparzeń, 1998; 9: 39-44.

25. Van Bebber PT, Lieners CF, Koldewijn EL, Redl H, Goris JA. Superoxide dismutase and catalase in an experimental model of multiple organ failure. J Surg Res, 1992; 52: 265-70.

26. Hongming Y, Zhiyong S, Zhenrong G, Zhiguo S, Jiangang L, Jiake C, Conpu S. Oxygen free radical injury and its relation to bacterial and endotoxin translocation after delayed resuscitation: clinical and experimental study. Chinese Med J, 1997; 110: 118-24.

27. Woolliscraft JO, Prasad JK, Thomson P, Till GO, Fox IH. Metabolic alterations in burn patients: detection of adenosine triphosphate degradation products and lipid peroxides. Burns, 1990; 16: 92-6.

28. Kumar R, Seth RK, Sekhon MS, Bhargava JS. Serum lipid peroxide and other enzyme levels of patients suffering from thermal injury. Burns, 1995; 21: 96-7.

29. Daryani R, Lalonde C, Zhu D, Weidner M, Knox J, Demling RH. Effect of endotoxin and a burn injury on lung and liver lipid peroxidation and catalase activity. J Trauma, 1990; 30: 1330-4.

30. Demling RH, Lalonde C. Systemic lipid peroxidation and inflammation induced by thermal injury persists into the post-resuscitation period. J Trauma, 1990; 30: 69-74.

31. Van Bebber PT, Boekholz WKF, Goris RJA, Schillings PHM, Dinges HP, Bahrami S, Redl H, Schlag G. Neutrophil function and lipid peroxidation in a rat model of Multiple Organ Failure. J Surg Res, 1989; 47: 471-5.

32. Gosling P, Sutcliffe AJ, Cooper MACS. Burn and trauma associated proteinuria: the role of lipid peroxidation, renin, myoglobin. Ann Clin Biochem, 1988; 25: 53-9.

33. Matsuda T, Tanaka H, Yuasa H. The effects of high dose vitamin C therapy on postburn lipid peroxidation. J Burn Care Rehabil, 1993; 14: 624-9.

34. Demling RH, Ikegami K, Lalonde C. Increased lipid peroxidation and decreased antioxidant activity correspond with death after smoke exposure in the rat. J Burn Care Rehabil, 1995; 16: 104-10.

35. Haycock JW, Ralston DR, Morris B, Freelander E, Mac Neil S. Oxidative damage to protein and alterations to antioxidant levels in human cutaneous thermal injury. Burns, 1998; 23: 533-40.

36. Redl H, Gasser H, Schlag G, Marzi I. Involvement of oxygen radicals in shock related cell injury. Brit Med Bull, 1993; 43: 556-65.

37. Haglund U, Gerdin B. Oxygen free radicals (OFR) and circulatory shock. Circ Shock, 1991; 34: 405-11

38. Hansbrough JF, Zapata-Sirvent RL, Peterson UM. Immunomodulation following burn injury. Chest Surg Clin N Am, 1987; 67: 15-29.