Effect of Varied Doses of Ascorbic Acid in Burn Patients: A Randomized Controlled Study

ABSTRACT

Background: Free radical-mediated systemic response with fluid and protein leakage from increased capillary permeability is a common feature of oxidative stress in major burn. This work sought to use quantitative urinary protein estimation and serum malondialdehyde to assess the antioxidant efficacy of varied doses of ascorbic acid in burn patients.

Materials and Methods: In this double-blind, randomized, controlled prospective study consecutive patients with major burn trauma presenting within 48 hours of the incident at the regional burn centre of National Orthopaedic Hospital, Enugu were recruited. Each burn patient in the treatment group received intravenous ascorbic acid, 2mg/kg/hour or 8mg/kg/hour over 24 hours in lactated Ringers resuscitation fluid. Each patient in the placebo group received 120 mls of Normal saline, in lactated Ringers resuscitation fluid. Oxidative stress status was determined in each patient by quantitative determination of the urinary protein concentration and serum malondialdehyde. Differences were considered significant when *P*< 0.05.

Results: Twenty-nine out of the forty-eight recruited burn patients had complete data for analysis. The mean age of these patients was 34.6±16.6 years, with mean percentage 'Total Burn Surface Area' of 43.7±18.5. Mean decrease in serum malondialdehyde was highest for patients on 8mg/kg/hour of ascorbic acid and the difference was found to be statistically significant, (*P*<0.001). Similarly, the mean decrease in urinary protein was highest for patients in the 8mg/kg/hour of ascorbic acid group and least for those in the placebo group, (*P*=0.013). There was no statistically significant correlation between the serum malondialdehydeor urinary protein concentration at presentation and the burns surface area or the patient's age.

Conclusion: Ascorbic acid at a dose of 8mg/kg/hour over 24 hours was superior to 2mg/kg/hour and placebo, in reducing oxidative stress indicators in patients with major burn.

Keywords: Adjuvant, ascorbic acid, burn, free radical, oxidative stress, resuscitation.

1. INTRODUCTION

Burn is commonly sustained from domestic fires, work-related accidents, assault, suicide, and in combat casualty. Morbidity and mortality attributed to burn injury have remained worrisome, even in highly-specialized burn centres [1]. A recent retrospective study undertaken in the sub-region similarly reported that mortality rate was 23.4%, with an admission rate of 69.5% for burn injury [2]. Much of the injury suffered by patients with burn and major trauma is attributable to oxidative stress and systemic response induced by reactive oxygen species (ROS) and reactive nitrogen species (RNS) [3, 4]. Following acute burn there is increased production of ROS mainly from raised xanthine oxidase activity. The host antioxidant systems act by preventing the formation of ROS through mechanisms such as the inhibition of enzymes that catalyze ROS formation, scavenging formed ROS, or by promoting their rapid decomposition. Whenever there is imbalance and preponderance of the production of these reactive species over the ability of the host anti-oxidant protective system to detoxify them, oxidative injury to the cells is the consequence; a condition termed "oxidative stress".

Direct quantification of the ROS which are responsible for oxidative stress is a major challenge on account of their very short half-life and extremely low concentrations in biologic systems. Despite recent success with direct quantification of ROS formation by techniques such as the

Electron spin resonance spectroscopy in experimental studies, there are still limitations with their application [5, 6]. Consequently, the mainstay of oxidative stress estimation has been the use of "fingerprint assays" which quantify the ROS-mediated damages on lipids [7], proteins [8] or nucleic acid molecules [9].

Lipid peroxidation is oxidative damage to lipids and represents a major mechanism of cell damage by ROS with the production of aldehydes such as malondialdehyde [MDA] and 4-hydroxynonenal [HNE] [10], among others. MDA has been widely used as a biomarker for lipid peroxidation because of its simple reaction with thiobarbituric acid [TBA] to yield a colouredchromogen fluorescent red adduct. The TBA assay is the most common and easiest method used as an indicator of lipid peroxidation and free radical activity in biological samples.

Proteinuria frequently accompanies the free radical-mediated vasculopathy in major burn, with the degree of proteinuria considered an indicator of the severity of the generalized capillary leakage. Burn-related proteinuria complicates the outcome, and has been associated with increased risk for developing acute kidney injury and mortality [11].

Antioxidant mineral depletion is a feature of major burn [12]. There is evidence that burn patients treated with antioxidants had significantly lower levels of inflammatory and immunological mediators at various times following the burn injury [13]. Ascorbic acid (vitamin C) is a relatively cheap but powerful antioxidant which is increasingly used for this indication in several clinical conditions, including major burn. Evidence indicates that therapy with high-dose intravenous ascorbic acid in the early phase of burn resuscitation improves fluid requirement and other outcome indices [14]. The safety of high- dose infusion of vitamin C was supported by clinical studies on burn patients, indicating absence of renal complication following therapy with infusion as much as 66mg/kg/hr [15, 16]. Despite this apparent safety profile case reports of acute renal failure from oxalate nephropathy have been documented in patients receiving high doses of vitamin C [17].

The aim of the study was to evaluate the antioxidant effect and safety of varied doses of vitamin C in reducing oxidative stress in patients with major burn.

MATERIALS AND METHODS
2.1 Study design: This is a double-blind, placebo-controlled clinical study.
2.2 Study setting
The burns section of the trauma unit, regional burns and trauma centre, National Orthopaedic Hospital, Enugu.
2.3 Study site
This study was conducted at the regional burns and trauma centre of the National Orthopaedic Hospital, Enugu. The hospital is a tertiary federal facility that serves as a referral centre for burns and other trauma in the Southeast geopolitical zone of Nigeria. In addition, diverse orthopaedic, plastic and reconstructive procedures are routinely provided at primary, secondary or tertiary care level. Many patients also access care in the facility from the North -central and South-south geopolitical zones, and as far as the Republic of Cameroon.

2.4 Sample size determination:
Derivation of sample size for quantitative variable in clinical intervention [18],

$$= \frac{2SD^2\ (Z\alpha/2 + Z\beta)^2}{d^2}$$

where SD – Standard deviation from previous study [19]

$Z\alpha/2 = Z0.05/2 = Z0.025 = 1.96$ (From Z table) at type 1error of 5%
$Z\beta = Z0.20 = 0.842$ (From Z table) at 80% power
d = projected effect size of 0.4 nmol/mL
$$= \frac{2(0.34)^2 (1.96+0.84)^2}{(0.4)^2} = \frac{2\times0.12 \times 7.84}{0.16} = 11.8 = 12 \text{ burn patients/ group}$$

Adjusted sample size. In making provision for potential attrition, assuming a dropout rate of 10%, the adjusted sample sizeis derived as; calculated sample size/ (1- dropout rate) [20]. Therefore, Adjusted sample size = 12/ (1-0.1) = 13.3 =13.
Therefore, the minimum sample size is 13 patients each, for the three groups.

2.5 Inclusion criteria
All the consenting burn patients with total burns surface area [TBSA] of 20%, or more, were considered for inclusion in the study.

2.6 Exclusion criteria
Patients were excluded if the burn severity was such that the patient could not be weighed on the 'standing scale'. 'Bed weighing scale' which is recommended for such frail patients is unavailable in our health facility.Patients with history of other independent causes of oxidative stress will also be excluded. These may include patients with epilepsy, obesity, diabetes mellitus, hypertension, rheumatoid arthritis, alcoholics, cigarette smokers, or patients on certain drug therapy (azidothymidine, cisplatin, doxorubicin, diclofenac, antidepressants, anticonvulsants, paracetamol, fluoroquinolones and chlorpromazine). Children below 12 years of age, patients with known renal impairment, patients whose burn injury is less than 20% TBSA and those patients presenting later than 48hours after the burn incident were also excluded.

2.7 Recruitment and randomization of the study sample population
The sample was recruited between May and October, 2023. All patients with major burn of any type who presented at the regional burn centre within 48 hours of the burn incident were considered. The patients were recruited consecutively on presentation at the burn facility. A questionnaire was used to implement the exclusion criteria. The recruited patients were evenly randomized into three groups; two test groups and a placebo group.

2.8 Blinding technique
The research assistant (the trauma physician in this instance) assisted in this collaboration, with administration of the medication for the burn patients. The burn patients were randomized to the three (3) groups by a lot of coloured cards; 16 green, 16 blue, and 16 white (representing placebo 'group 1', 8mg/kg/hour of intravenous vitamin C 'group 2', and 2mg/kg/hour vitamin C 'group 3', respectively). While the investigator who picked the cards for group allocation did not know the category of each colour-code; which is known only to the research assistant, the latter did not partake in collecting the blood samples for the laboratory estimations.

2.9 Specimen collection and intervention
Blood and urine samples were collected with plain specimen bottles and sterile universal bottles respectively, from all the consenting burn patients for the estimation of pre-intervention serum malondialdehyde and total urinary protein. Serum electrolytes, urea and creatinine were also determined from the obtained serum sample to assess each patient's renal status. These patients were now randomly allocated to the three groups. The placebo group received Ringers lactate infusion as resuscitation fluid plus 24 × 10mls volumes of Normal saline infusion injected into the various resuscitation fluid bags over 24hours (Group 1), while the test groups received Ringers lactate infusion as resuscitation fluid plus 8mg/kg/hour of vitamin C injection (Group 2), and 2mg/kg/hour of vitamin C injection (Group 3) injected into the various resuscitation bags

over 24 hours. The vitamin C injection that was used is 500mg/10ml ampoule. Thus, a 70kg burn patient in the Group 2 received 26 ampoules of vitamin C injection containing 13g of vitamin C. The solution bags were covered with a black cellophane bag to prevent light-induced auto-oxidation of vitamin C. In each patient a repeat estimation of the oxidative indicators and renal status was obtained 48hrs after completion of the interventional therapy.

2.10 Biochemical analysis

Oxidative stress evaluation was based on quantitative measurement of total protein concentration in the urine sample, and lipid peroxidation product (malondialdehyde) in the serum of the patients. These parameters were re-evaluated 48 hours post-intervention.

2.10.1 Determination of serum MDA level

The serum malondialdehyde was determined using the 'modified thiobarbituric acid reactive substance 'TBARS' technique, as determined by spectrophotometry [21].

Principle: Malondialdehyde (MDA) is a product of lipid peroxidation. When heated with 2-thiobarbituric acid (TBA) under alkaline condition, it forms a pink coloured product, which has absorption maximum at 532 nm. The intensity of colour generated is directly proportional to the concentration of MDA in the sample.

2.10.2. Determination of urine protein concentration

The quantitative determination of protein in the urine was accomplished by 'sulphosalicylic acid technique', as determined by spectrophotometry [22].
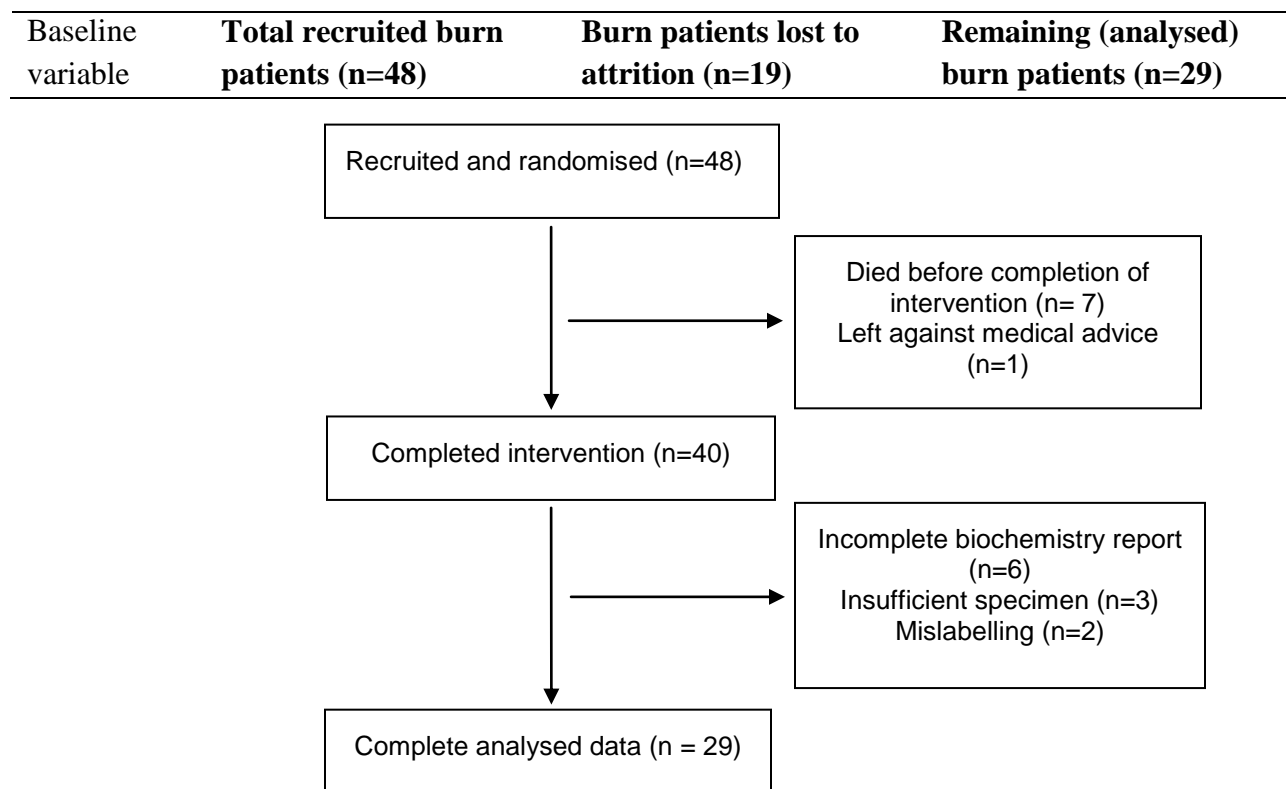
Principle:

The acidification of urine by sulphosalicylic acid causes precipitation of protein in the sample seen as turbidity and the degree of turbidity is directly proportional to the concentration of protein present in the urine sample.

2.11 Statistical analysis of data

Data entry and analysis were done using IBM Statistical Product for Service Solutions (SPSS) statistical software version 25. Categorical variables were summarized using frequencies and proportions while continuous variables were presented using mean and standard deviation but median was used when the data was skewed. Chi square test of statistical significance was used to compare the difference in proportions between categorical variables. Correlation analysis was used determine the strength of linear relationship between two continuous variables. Analysis of variable was used to compare the mean of more than two groups and when the data was skewed, Kruskal Wallis test was used. In all applications of these statistical tests, the level of statistical significance was determined by a P value of < 0.05.

2.12 CONSORT flow diagram

Baseline variable	Total recruited burn patients (n=48)	Burn patients lost to attrition (n=19)	Remaining (analysed) burn patients (n=29)

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        Recruited and randomised (n=48)
                      |
                      |---------------------->  Died before completion of
                      |                          intervention (n= 7)
                      |                          Left against medical advice
                      |                          (n=1)
                      v
        Completed intervention (n=40)
                      |
                      |---------------------->  Incomplete biochemistry report
                      |                          (n=6)
                      |                          Insufficient specimen (n=3)
                      |                          Mislabelling (n=2)
                      v
        Complete analysed data (n = 29)
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3. RESULTS

Forty-eight patients were recruited for the study. Owing to high attrition complete data was available for only twenty-nine of the burn patients and were analysed accordingly. The mean age of these patients was 34.6±16.6 years (range; 20- 73 years), with a predominance of females; 72% (21/29). The mean % TBSA was 43.7±18.5 (range; 20-85%).

Table 1 shows the baseline characteristics of the entire sample of recruited burn patients, including those that didn't complete the intervention, completed but had had incomplete data, and those who completed the study with intact data for analysis. The burn patients with incomplete data appear to be comparatively older than those with complete and analysed data.

Table1: Baseline characteristics of the recruited sample of burn patients.

Sample composition	Group 1 (n=16)	Group 2 (n=16)	Group 3 (n=16)	Group 1 (n=6)	Group 2 (n=6)	Group 3 (n=7)	Group 1 (n=10)	Group 2 (n=10)	Group 3 (n=9)
Gender									
Male	5	6	5	2	3	3	3	3	2
Female	11	10	11	4	3	4	7	7	7
Age of the patients (yrs)									
Mean±SD	37.3± 20.9	39.6± 16.2	39.1± 15.1	42.8± 21.9	44.7± 19.2	46.9± 11.5	33.9± 20.7	36.6± 14.4	33.1± 15.4
%TBSA									
Mean±SD	49.5± 18.2	50.6± 16.0	42.6± 21.5	46.8± 14.0	55.8± 16.8	57.4± 21.9	51.4± 20.9	47.5± 15.5	31.1± 12.7

Table 2 shows the comparison of participants' characteristics. The mean age of participants in the three groups was comparable, (P=0.898). The mean percentage burns of participants in placebo group was the highest, 51.4±20.9 while that of those in lower dose ascorbic acid was the least, 31.1±12.7 and the difference in mean was found to be statistically significant, (F=3.822, P=0.035).

Table 2: Comparison of the patients' characteristics of the three groups of burn patients that were analysed.

Variable	Group 1 (n=10)	Group 2 (n=10)	Group 3 (n=9)	Test statistics	*P* value
Age of the patients in years					
Mean±SD	33.9±20.7	36.6±14.4	33.1±15.4	0.110*	0.898
Gender					
Male	3 (30.0)	3 (30.0)	2 (22.2)	0.188**	0.910
Female	7 (70.0)	7 (70.0)	7 (77.8)		
%TBSA					
Mean±SD	51.4±20.9	47.5±15.5	31.1±12.7	3.822 ***	0.035

*Kruskal Wallis, **Chi square test   ***Analysis of variance

Table 3 shows the intergroup comparison of the patient parameters, before and after treatment. The mean pre-treatment malondialdehyde was highest among participants in higher dose ascorbic acid group, 2.5±0.6 nmol/mL and was least in the placebo group, 1.4±0.4 nmol/mLand the difference in mean was found to be statistically significant, (F=15.954, *P*<0.001). The mean post-treatment malondialdehyde for the three groups was comparable, (F=0.927, *P*=0.408). The mean pre-treatment and post-treatment proteinuria for the three groups were comparable, (Pre-treatment, F=3.170, *P*=0.059) and (Post-treatment, F=2.226, *P*=0.128).

Table 3: Intergroup comparison of the outcome parameters, before and after treatment.

Variable	Group 1 (n=10)	Group 2 (n=10)	Group 3 (n=9)	F	*P* value
Pre-treatment MDA (nmol/mL)					
Minimum	1.1	1.9	1.0	15.954	<0.001
Maximum	2.2	3.5	2.3		
Mean±SD	1.4±0.4	2.5±0.6	1.5±0.5		
Post-treatment MDA (nmol/mL)					
Minimum	1.0	1.0	0.9	0.927	0.408
Maximum	3.0	2.1	2.0		
Mean±SD	1.6±0.6	1.5±0.3	1.4±0.3		
Pre-treatment proteinuria (mg/L)					
Minimum	203.0	190.0	237.0	3.170	0.059
Maximum	320.0	490.0	407.0		
Mean±SD	245.3±38.9	327.0±108.3	315.6±70.5		
Post-treatment proteinuria (mg/L)					
Minimum	206.0	186.0	227.0	2.226	0.128
Maximum	307.0	280.0	340.0		
Mean±SD	243.1±34.2	224.8±37.3	259.9±37.2		

Table 4 shows within-group comparison of mean malondialdehyde and proteinuria among the study groups. The mean malondialdehyde for participants in Group 2 was significantly higher at baseline, 2.5±0.6 when compared with post intervention values, 1.5±0.3, (paired t test=6.943, *P*<0.001). Similarly, the mean proteinuria for participants in Group 2 was significantly higher at

baseline, 327.0±108.3 when compared with post intervention values, 224.8±37.3, (paired t test=3.255, P=0.010). Also, the mean proteinuria for participants in Group 3 was significantly higher at baseline, 315.6±70.5 when compared with post intervention values, 259.9±37.2, (paired t test=2.960, P=0.018).

Table 4: Within-group comparison of mean malondialdehyde and proteinuria among the study groups.

Variable	(MDA)		Paired t test P value	Proteinuria		Paired t test P value
	Before Intervention	After Intervention		Before Intervention	After Intervention	
Group 1 Mean±(SD)	1.4±0.4	1.6±0.6	1.300 P=0.226	245.3±38.9	243.1±34.2	0.190 P=0.853
Group 2 Mean±(SD)	2.5±0.6	1.5±0.3	6.943 P<0.001	327.0±108.3	224.8±37.3	3.255 P=0.010
Group 3 Mean±(SD)	1.5±0.5	1.4±0.3	1.394 P=0.201	315.6±70.5	259.9±37.2	2.960 P=0.018

Table 5 shows that the mean decrease in serum MDA was highest for patients on higher dose ascorbic acid (1.0±0.50 nmol/mL) and the difference in mean was found to be statistically significant, (P<0.001). Similarly, the mean decrease in urinary protein was highest for patients in higher dose ascorbic acid group, (102.2±99.3 mg/L) and least for those in the placebo group, (2.2±36.5 mg/L) and the difference in mean was found to be statistically different, (P=0.013).

Table 5: Comparison of the effect of the treatment on MDA and urinary protein in the three groups

Variable	Group 1 (n=10)	Group 2 (n=10)	Group 3 (n=9)	Kruskal Wallis	P value
Change in malondialdehyde (nmol/mL)					
Mean±SD	0.2±0.5	1.0±0.5	0.2±0.4	18.662	<0.001
Median	0.04	1.0	0.1		
Change in					

proteinuria (mg/L)

Mean±SD	2.2±36.5	102.2±99.3	55.7±56.4	5.159	0.013
Median	4.0	88.5	37.0		

Figure 1 reveals a marked decrease in serum MDA post-treatment in group 2, while only a slight decrease and an increase in serum MDA were observed in groups 3 and 1, respectively.

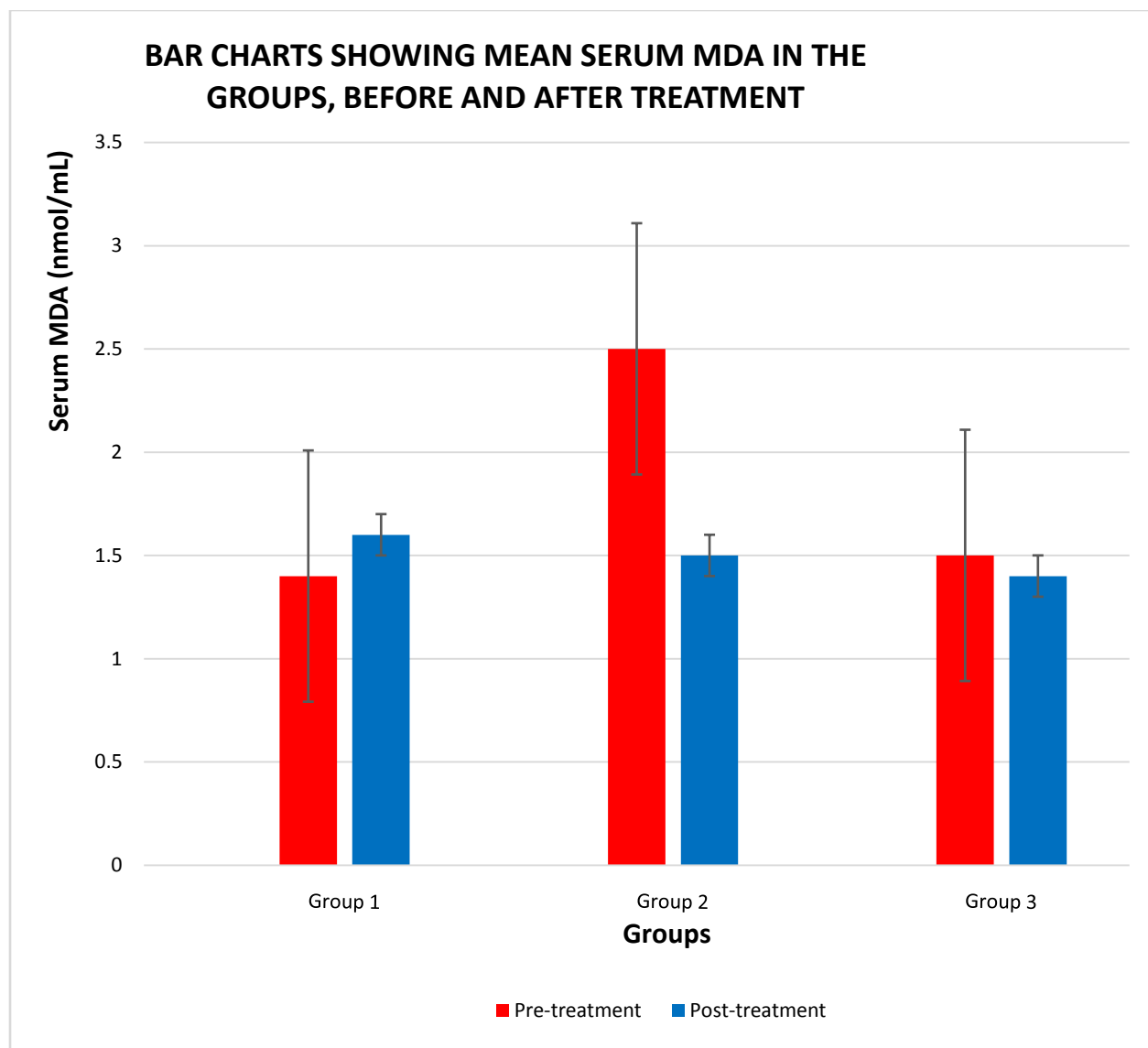**Figure 1: Bar charts showing mean serum MDA in the groups, before and after treatment**

BAR CHARTS SHOWING MEAN SERUM MDA IN THE GROUPS, BEFORE AND AFTER TREATMENT

Figure 2 shows that the greatest reduction in urinary protein was observed in the burn patients in group 2, while the level of proteinuria in group 1 burn patients was almost unchanged.

Figure 2: Bar charts showing mean urinary protein in the groups of burn patients, before and after treatment.
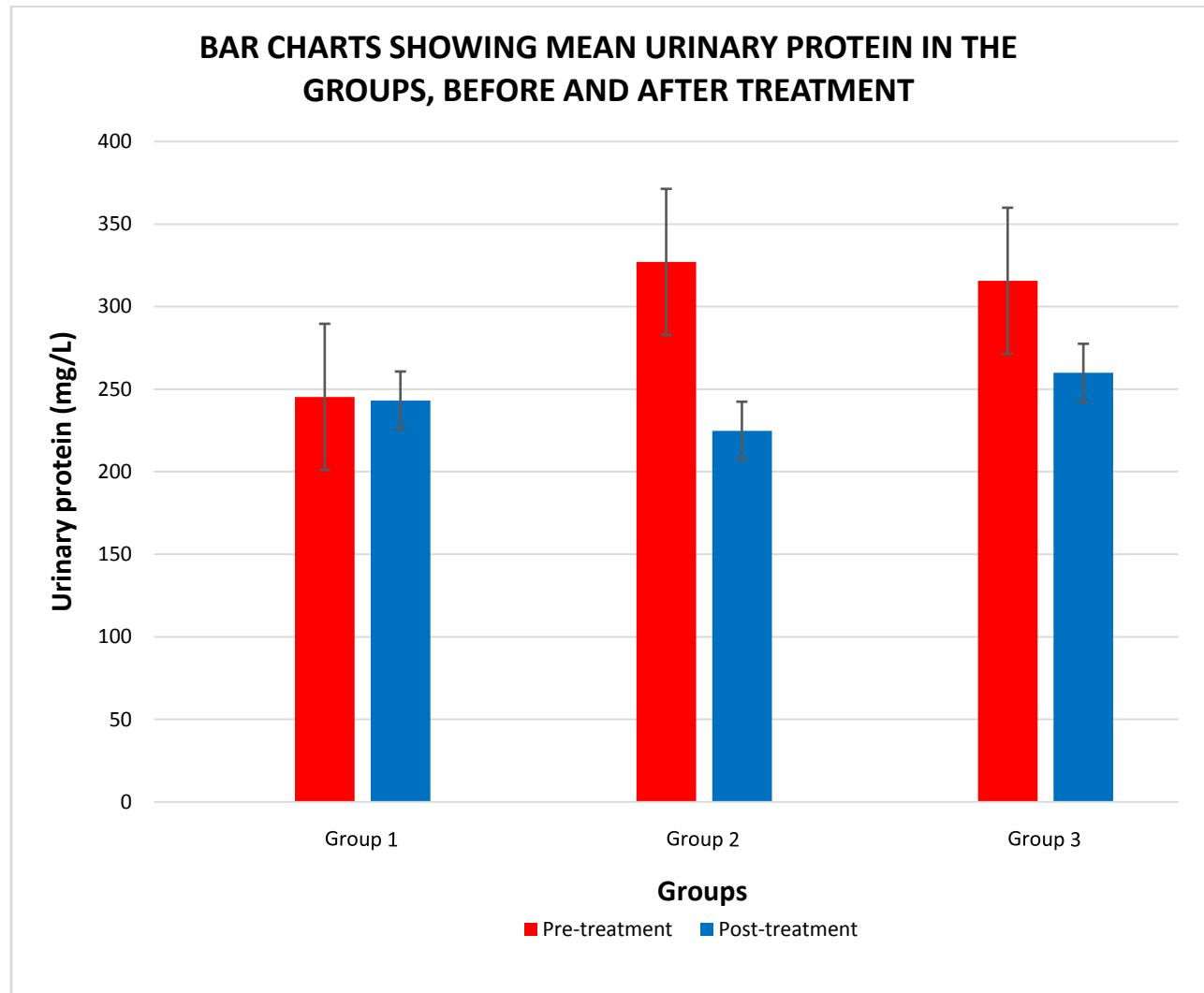

BAR CHARTS SHOWING MEAN URINARY PROTEIN IN THE GROUPS, BEFORE AND AFTER TREATMENT

Table 6 shows the comparison of participants' characteristics with pre-treatment MDA and proteinuria. There was a strong negative correlation between serum MDA and age of the burn patients in years for those in placebo group, such that increase in age correlates with decrease in serum MDA but this was not found to be statistically significant, (n=10, r=-0.605, P=0.064). There was a weak negative correlation between serum MDA and % TBSA for those in low dose ascorbic acid group, such that increase in percentage burns correlates with decrease in serum MDA but this was not found to be statistically significant, (n=9, r=-0.233, P=0.564). There was a weak positive correlation between urinary protein and %TBSA for those in high dose ascorbic acid group, such that increase in percentage burns correlates with increase in urinary protein but this was not found to be statistically significant, (n=10, r=0.210, P=0.560).

Table 6: Correlation between the patients' characteristics and the outcome variables, at presentation.

Variable	Sample size (n)	Correlation coefficient (r)	*P* value
Group 1			
Correlation of MDA with			
Age in years	(n=10)	-0.605	0.064
% TBSA	(n=10)	-0.271	0.450
Group 2			
Correlation of MDA with			
Age in years	(n=10)	-0.315	0.376
% TBSA	(n=10)	-0.175	0.629
Group 3			
Correlation of MDA with			
Age in years	(n=9)	0.315	0.409
% TBSA	(n=9)	-0.223	0.564
Group 1			
Correlation of urinary protein with			
Age in years	(n=10)	-0.322	0.364
% TBSA	(n=10)	0.084	0.817
Group 2			
Correlation urinary protein with			
Age in years	(n=10)	0.421	0.226
% TBSA	(n=10)	0.210	0.560
Group 3			
Correlation of urinary protein with			
Age in years	(n=9)	-0.238	0.538
% TBSA	(n=9)	-0.432	0.245

Table 7 shows the comparison of the serum chemistry in the burn patients, at presentation and following treatment. The pre-treatment mean serum creatinine level was highest in the placebo group, 1.7±1.6 mg/dL but the difference in mean compared to the other groups was not statistically significant (F=1.154, P=0.331).The post-treatment mean creatinine value was highest among participants in the placebo group, 1.3±1.2 mg/dL but the difference in mean was not found to be statistically significant, (F=1.296, P=0.291). Two patients developed raised serum creatinine exceeding 0.3mg/dL over the 48 hr period of the treatment intervention; one from 0.9mg/dL to 2.3 mg/dL, while the other had a serum creatinine elevation from 1.1 to 1.5mg/dL.

Table 7: Comparison of the serum chemistry in the burn patients, at presentation and following treatment.

Biochemical variable Mean±SD	Group 1 (n=10)	Group 2 (n=10)	Group 3 (n=9)	F	P value
Pre-treatment					
Sodium (mmol/L)	132.0±5.0	129.1±9.2	136.6±11.3	1.715	0.200
Potassium (mmol/L)	4.6±0.9	4.1±0.8	9.4±16.4	0.958	0.397
Chloride (mmol/L)	95.9±6.3	102.2±6.1	96.1±4.5	3.822	0.035
Bicarbonate (mmol/L)	17.0±4.1	19.8±2.0	20.7±5.4	2.202	0.131
Urea (mg/dL)	43.3±31.7	28.6±21.1	29.0±23.5	1.027**	0.372
Creatinine (mg/dL)	1.7±1.6	1.0±0.6	1.1±0.5	1.154**	0.331
Post-treatment					
Sodium (mmol/L)	130.5±7.5	132.2±4.9	133.9±3.7	0.858	0.436
Potassium (mmol/L)	3.9±0.7	3.7±0.6	3.8±0.2	0.147	0.864
Chloride (mmol/L)	93.8±4.0	95.7±7.6	93.9±5.1	0.337	0.717
Bicarbonate (mmol/L)	21.2±5.3	22.7±2.5	24.4±1.9	1.919	0.167
Urea (mg/dL)	44.5±45.3	33.7±24.6	20.3±15.3	1.394**	0.266
Creatinine (mg/dL)	1.3±1.2	1.0±0.5	0.7±0.3	1.296	0.291

**Kruskal Wallis

4. DISCUSSION
The burn patients that received 8mg/kg/hr of ascorbic acid recorded significantly greater decreases in the serum malondialdehyde than those who received 2mg/kg/hr and those treated with normal saline placebo. Similarly, there was marked decrease in proteinuria in the group of burn patients treated with 8mg/kg/hr of ascorbic acid compared to the other two groups.
The mean serum MDA in Groups 1, 2 and 3 at presentation were 1.4±0.4 nmol/mL, 2.5±0.6 nmol/mL, 1.5±0.5 nmol/mL respectively. A study conducted in Iraq had reported a mean serum

MDA of 0.81±0.16 nmol/mL in healthy volunteers [19]. The researchers had measured the serum MDA levels using the standard methods of Stocks and Dormandy as modified by Gilbert et al. [23]. In another study conducted in India, the mean serum MDA of healthy volunteers was given as 0.93±0.39 nmol/mL [24]. These researchers estimated MDA in serum by using trichloroacetic acid and 1% thiobarbituric acid (TBA). Both techniques employed in the aforementioned studies are variants of the TBARS analytical method used in our study. Various modifications of the TBARS-based MDA estimation have been adopted by different researchers in a bid to limit undesirable interference and minimize analytical errors on the MDA quantification. At presentation, the mean serum MDA of all three groups of our burn patients were higher than the values reported for healthy volunteers in the referenced studies above and lend support for the greater oxidative stress imposed by burn injury. However, it has been suggested that the timing of MDA estimation following burns could remarkably impact on the measured serum levels. The Iraqi study measured the serum MDA of their burnt patients within 24hr post-burn and reported markedly elevated levels; 5.54±0.34 nmol/mL, 6.1±1.1 nmol/mL, 5.95 ±1.5 nmol/mL, 5.7 ±0.9 nmol/mL, 4.86 ±0.7 nmol/mL, 6.2±1.1nmol/mL, for the various groups that were to receive different antioxidant interventions [19].These elevated levels were recorded in contrast to a mean serum MDA of 0.81±0.16 nmol/mL in their healthy volunteers [19]. The temporal course of serum MDA levels in burn patients has been revealed in earlier studies, indicating a steady decline over time [15, 25]. Most of the patients in the present study had delayed presentation. The importance of temporal consideration while measuring serum levels of MDA in burn patients has led to an earlier recommendation for its investigation in oxidative stress studies involving this patient population [25].

The greatest reduction in urinary protein was observed in the burn patients treated with 8mg/kg/hr of ascorbic acid. The group of burn patients treated with 2mg/kg/hr of ascorbic acid recorded less remarkable reduction in proteinuria while the level of proteinuria in the burn patients treated with normal saline placebo was almost unchanged.

This study however revealed no significant correlation between the % TBSA and serum MDA of the burns patients at presentation. The finding is supported by other authors that reported the absence of correlation between burn severity at presentation and the levels of lipid peroxidation products in the patients [26, 27]. This may suggest that other parameters of burn trauma such the thickness of the burn, type of burn or presence of inhalation injury may be a better indicator of severity of oxidative stress. Furthermore, there was no significant correlation between the participants' age and serum MDA or degree of proteinuria.

Acute kidney injury (AKI) is not an uncommon complication of major burn, often arising from severe hypovolaemia, acute haemolysis, rhabdomyolysis and myoglobinuria. Acute kidney injury contributes significantly to mortality and morbidity in major burn. According to the Kidney Disease Improving Global Outcomes (KDIGO) guidelines AKI may be defined by increase in serum creatinine by ≥0.3 mg/dL within 48 hours, or increase in serum creatinine to ≥1.5 times baseline, which is known or presumed to have occurred within the prior seven days, or urine volume <0.5 mL/kg/hour for six hours [28]. By this definition two burn patients in Group 2 (a 22 year old female with 25% TBSA, and a 37 year old male with 73% TBSA) developed AKI. However, since the baseline serum creatinine of the patients prior to the burn trauma was unknown in the burn patients the incidence of AKI based on that criterion may have been grossly understated. With the elevated levels of creatinine at presentation many of the burn patients may well have developed AKI prior to the initial measurement. The requirement to

compare the supposedly elevated serum creatinine with a prior baseline level which is usually unknown at presentation similarly encumbers the definition of AKI caseness based on the popular 'Risk, Injury, Failure, Loss of kidney function and End-stage kidney disease' (RIFLE) classification [29]. This confounding shortcoming with the diagnostic criteria and the multiplicity of definitions applied for AKI have been highlighted in a previous review [30]. Whether the AKI in the two patients arose from the burn injury itself or iatrogenic complication of the dose of 8mg/kg/hr of ascorbic acid could not be ascertained. High-dose ascorbic acid therapy has rarely been implicated in AKI from acute oxalate nephropathy in burn patients and other clinical conditions [31, 32].

There was a preponderance of females among the recruited burn patients. This trend persisted in the three groups of burn patient in the analysed data. In this regard our patient cohort contrasts with data from other burn patient cohorts and epidemiological studies which report a preponderance of males in burn trauma [2, 25, 33].Meanwhile evidence indicates that the female gender propagates less vigorous oxidative stress response than males and correspondingly better antioxidant response [34].Consequently, howthe disproportionate female representation in our cohort could have impacted the results of the study is subject to conjecture.

4.1 Attrition bias
Nineteen out of the 46 recruited patients (39.6%) had incomplete data amounting to high attrition in excess of the 10% permitted for the study. The impact of such high attrition rate on the validity of a study such as this is well-known [35, 36].

4.2 Error bars
There were remarkably long error bars in the bar charts for the pretreatment serum MDA, and even the pretreatment urinary protein in the three groups of patients evaluated in the study. This is indicative of the wide variability in these outcome variables at presentation, the calculated mean values notwithstanding.

4.3 Limitations
The % TBSA in the three groups of patients at presentation were not comparable, and could have impacted on the outcome despite the lack of significant correlation between %TBSA and the outcome variables of MDA and urinary protein in the individual groups. It is reasonable to infer that the variability in the methods of MDA estimation adopted by different researchers could impair efforts at direct comparison of results. Furthermore, the high level of attrition resulting in suboptimal number of burn patients with complete data could have reduced the statistical power of the study. On its own, the high attrition rate, whether random or systematic carries potential error of bias that could impair the validity of the findings.

5. CONCLUSION
Intravenous ascorbic acid adjuvant therapy at a dose of 8mg/kg/hr was efficacious in reducing oxidative stress indicators in burn patients. This dose significantly reduced the serum malondialdehyde level and urinary protein excretion in burn patients more than the dose of 2mg/kg/hr and Normal saline placebo.

Ethical approval and consent
The study protocol was approved by the Research Ethics Committee of the National Orthopaedic Hospital, Enugu. Written informed consent was sought and obtained from each patient, or

parent/guardian in the case of minors, before recruitment into the study. The study was conducted in compliance with the Consolidated Standards of Reporting Trails (CONSORT) guidelines, aided by a CONSORT flow chart.

Conflict of interest: The authors declare that no conflicting interest exists.

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