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Evaluation of Ascorbic Acid Adjuvant Therapy and Oxidative Stress Parameters in Burns Patients

A. D. G. Nwosu[1*], E. N. Ossai[2], O. Onwuasoigwe[3], F. N. Ahaotu[4], J. K. Anieze[5], E. I. Umeji[5], B. Okonedo[4] and O. Ogboji[4]

[1]Department of Anaesthesia. National Orthopaedic Hospital, Enugu, Nigeria.
[2]Department of Community Medicine, College of Health Sciences, Ebonyi State University, Abakaliki, Nigeria.
[3]Department of Orthopaedics, UNTH Ituku-Ozalla, Enugu, Nigeria.
[4]Department of Orthopaedics, National Orthopaedic Hospital, Enugu, Nigeria.
[5]Department of Plastic Surgery, National Orthopaedic Hospital, Enugu, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors ADGN and ENO provided study concept, design, definition of intellectual content, data acquisition, data analysis, statistical analysis, prepared and revised the original draft of the manuscript. Authors OO, JKA, FNA, EIU, BO and OO designed the study, definition of intellectual content, data analysis, manuscript revision. All authors approved the final draft of the manuscript.

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ABSTRACT

Background: Burns trauma is associated with considerable morbidity and mortality. Resuscitation of burns patients with high-dose of Ascorbic acid has shown potential for mitigating the injury, but the optimal dose for this indication is unknown.
Aim: This study aimed to evaluate the impact of adjuvant therapy with intravenous Ascorbic acid (6g over 24 hours) on indicators of oxidative stress in patients with major burns.
Materials and Methods: We conducted a randomized placebo-controlled study on patients with major burns who presented at the National Orthopaedic Hospital, Enugu, Nigeria between August 2017 and July 2020. Each patient in the treatment group received intravenous Ascorbic acid, 6g over 24 hours, while those in the placebo group received Normal saline in the resuscitation fluid.

**Corresponding author: E-mail: adnwosu@yahoo.com;*

Oxidative stress evaluation was based on measurement of total antioxidant capacity and malondialdehyde in the participants. The level of statistical significance was determined by a p value of <0.05.

Results: The study was conducted on 37 burns patients and 15 healthy subjects. At presentation, the burns patients had significantly lower total antioxidant capacity; *P*=0.006, and higher serum malondialdehyde; *P*=0.040, compared to the healthy volunteers. The decrease in serum malondialdehyde in the burns patients treated with high-dose Ascorbic acid; 0.9±0.8 nmol/mL, was greater than that in those treated with placebo; 0.3±1.4 nmol/mL. Similarly the increase in total antioxidant capacity in the burns patients treated with high-dose Ascorbic acid; 151.7±116.5 µmol/L was greater than that in those treated with placebo; 58.4±219.1 µmol/L. However these effects weren't statistically significant.

Conclusion: Intravenous Ascorbic acid at a dose of 6g over 24 hours did not significantly alter the indicators of oxidative stress in the burns patients, under the prevailing conditions of the study.

Keywords: Ascorbic acid; burns; oxidative stress; malondialdehyde; total antioxidant capacity.

1. INTRODUCTION

Burns trauma is associated with considerable morbidity and mortality, with non-fatal burns being a major cause of morbidity; with prolonged hospitalization, multiple surgeries, disfigurement, disability and stigma. Earlier, a 2005 study in our regional burns centre recorded 23.2% mortality among 285 burns patients [1]. Much of the injury suffered by patients with burns is attributable to oxidative stress induced by reactive oxygen species [ROS]. Generation of ROS which is dramatically increased in burns plays a role in propagating burn injury through oxidative damage to DNA, aminoacids and lipids, and the deactivation of enzymes [2,3].

Direct quantification of the ROS is a major challenge on account of their very short half-life and extremely low concentrations in biologic systems. The mainstay of oxidative stress estimation has been the use of "fingerprint assays" which quantify; (i) ROS-mediated damages on lipids, proteins or DNA molecules; and (ii) residual antioxidant concentration following ROS insult. 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]. Malondialdehyde has been widely used as a biomarker for lipid peroxidation because of its simple reaction with thiobarbituric acid [TBA] to yield a red adduct. Total antioxidant capacity (TAC) is a method of comprehensive assessment of different elements of antioxidant defense system. However owing to several shortcomings, information on TAC by itself alone is not sufficient to make inferences about oxidative stress [4,5]. It is thus recommended that a marker of antioxidant capacity should always be associated with a marker of oxidative damage when the aim is to make inferences about oxidative stress.

Resuscitation with intravenous high-dose of Ascorbic acid (vitamin C) has shown promise in reducing morbidity and mortality in burns patients, but the optimal dose and duration of the therapy remain unresolved. Over two decades ago, Tanaka et al. investigated the benefit of high-dose vitamin C; 66 mg/kg per hour (equivalent of 79g over 24hrs in a 50kg patient or 110g in a 70kg patient) in attenuating ROS-mediated oxidative injury in severely burnt patients [6]. The laudable outcome of this landmark clinical trial has been supported by other works [7,8]. However with the few reports of oxalate nephropathy and acute kidney injury following this remarkably high dose some valid concern remains over its safety [9]. Meanwhile it has not been proven that such a high dose is clinically necessary; and the reported effectiveness of intravenous vitamin C at 33mg/kg/hr (half the dose used by Tanaka et al.) in severely burnt patients by Quin et al. attests to this [10]. Indeed an animal study had revealed earlier that even mild dietary supplementation of vitamin C reduced peroxynitrite formation and atrial electrophysiological remodeling induced by rapid pacing in dogs [11]. In this study, we sought to evaluate the impact of adjuvant therapy with intravenous vitamin C (6g over 24 hours) on indicators of oxidative stress; serum malondialdehyde (MDA) and total antioxidant capacity (TAC) in patients with major burns.

2. MATERIALS AND METHODS

2.1 Study Design

This is a randomized double-blinded, placebo-controlled, clinical study.

2.2 Sample Size Determination

Formula for sample size calculation for quantitative data, in interventional studies [12];

$$\text{Sample size} = \frac{2SD^2(Z\alpha/2 + Z\beta)^2}{d^2} = \frac{2\times 0.2^2\times(1.96+1.282)^2}{0.25^2} = \frac{2\times0.04\times10.51}{0.0625} = 13.5.$$

Where SD is the standard deviation of the variable TAC (mmol/L) from a previous study [13], taken as 0.20 mmol/L
Zα/2 = 1.96 (from Z table, at type 1 error of 5%)
Zβ = 1.282 (from Z table, at 90% power)
d= effect size, or projected difference between mean values of the treatment and placebo groups taken as 0.25 mmol/L

Hence the minimum number of burns patients needed per group = 14. In making provision for possible attrition, additional 10% (1.4 patients) was granted; thus implying 15 burns patients per group.

2.3 Subjects and Methods

All consecutive, consenting adult burns patients with percentage total burns surface area [%TBSA] of 20% or more, who presented at the regional burns centre between August 2017 and July 2020 were considered for inclusion in the study. A questionnaire was used to implement the exclusion criteria. Among those excluded were subjects/patients with history of other independent causes of oxidative stress such as those with epilepsy, obesity, diabetes mellitus, hypertension, rheumatoid arthritis, alcoholics, cigarette smokers, or malignancies. Children, patients with renal impairment, patients whose burn injury was less than 20% TBSA and those patients presenting more than 48hours after the burn incident were also excluded. The selected, consenting healthy subjects who do not have known independent causes of oxidative stress mentioned above served as control for normal level of the measured oxidative stress indicators in the population.

2.3.1 Randomization and blinding technique

The research assistant (the trauma physician in this instance) assisted in this collaboration, with administration of the medications for the burns patients. The burns patients were randomized to either of the two groups by a lot of coloured cards; green and purple, representing either of the treatment or placebo groups. While the investigator who picks the cards for group allocation does not know the category of each colour code (which is known only to the research assistant), the latter does not partake in collecting the blood samples for the laboratory estimations. Thus both the investigator and the burns patients are blinded to the assigned test groups; which are known only to the research assistant administering the intervention.

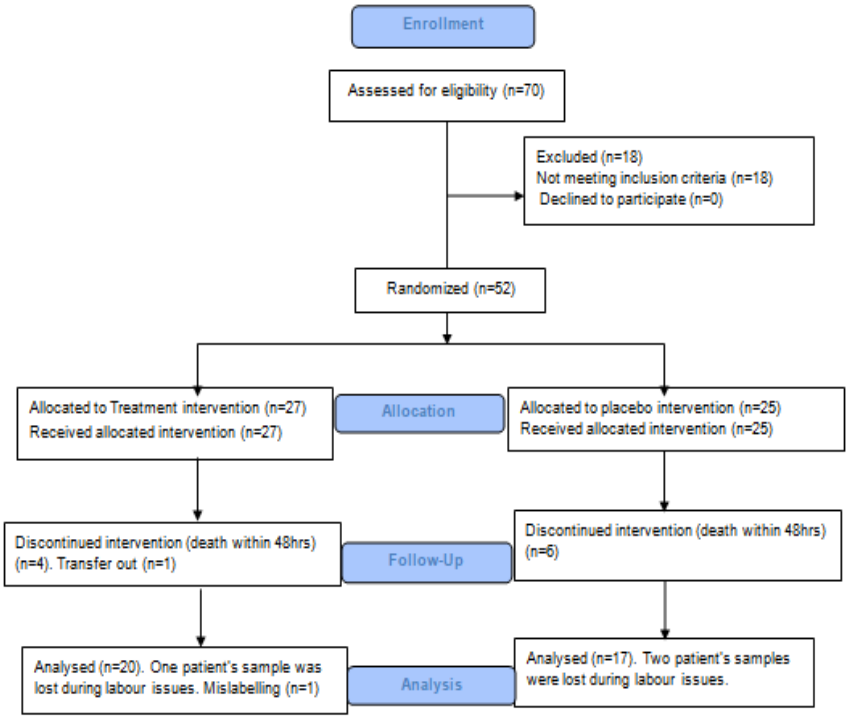
2.3.2 Fluid resuscitation

Fluid resuscitation was accomplished with Ringers lactate solution to restore circulatory volume and tissue perfusion with adequate urine output (0.5-1.0 ml/kg per hour), using the 'Parkland formula' [14] as a guide. The percentage total burns surface area (% TBSA) was estimated in each burns patient using the 'Wallace Rule-of-Nines'.

2.3.3 Sample collection and intervention

A venous blood sample was collected once from each of the healthy subjects for estimation of the oxidative indicators under investigation; they received neither Ringers lactate nor vitamin C infusion and no repeat estimation was required. Venous blood samples were also collected from all the selected, consenting burns patients to estimate the pre- intervention serum TAC and MDA. Their serum electrolytes, creatinine and urea were also determined to assess their pre-intervention renal status.The burnspatients were thereafter randomly allocated to either of groups I (Treatment group); and received Ringers lactate infusion as resuscitation fluid plus 12× 5mls volumes of vitamin C injection, injected into the various resuscitation bags over 24 hours, or group II (Placebo group) and received Ringers lactate infusionas resuscitation fluid plus 12× 5mls volumes of Normal saline infusion injected into the various resuscitation fluid bags over 24hours. In addition, all the burns patients (both treatment and placebo groups) received Ascorbic acid tablets (1g/day), vitamin E capsules (1000 iu/day) and vitamin A capsules (10,000 iu/day) as part of routine care. All the solution bags were covered with a

Consort 2010 Flow Diagram



black cellophane bag to prevent light-induced auto-oxidation of Ascorbic acid. In each burns patient a repeat estimation of the oxidative indicators and renal status was obtained 48hrs after commencement of the interventional therapy.

2.3.4 Serum preparation

The venous blood drawn from the participants into plain sample tubes were allowed for 30 minutes to clot and then centrifuged at room temperature for 5 minutes at 3000 rpm to extract the serum. While the electrolytes, urea and creatinine determination was processed as routine, the extracted sera for the determination of TAC and MDA were frozen and stored at -20°C, until assayed.

2.4 Measurements

Oxidative stress evaluation was based on measurement of the residual antioxidant status (total antioxidant capacity) and lipid peroxidation product (malondialdehyde) in the serum of the subjects. The parameters obtained from the healthy subjects served for comparison. The serum malondialdehyde level was determined by the method of Gutteridge and Wilkins [15], while total antioxidant activity was estimated by the ferric reducing ability of plasma (FRAP) method [16].

2.4.1 Equipment

Spectrophotometer (APAL PD303S, Japan), Incubator (MEMMERT, Germany), microplate reader (biobase 10b, China), Waterbath with shaker (ThermoScientific 2871, USA).

2.5 Data Presentation and Analysis

Data entry and analysis were done using IBM Statistical Package for Social Sciences (SPSS) version 25. Data were presented using tables and charts. Continuous variables were summarized using mean and standard deviation while categorical variables were summarized using frequencies and proportions. Chi square test was used to compare categorical variables. Correlation test was used to compare the strength of linear relationship between two continuous variables. Student t test, Man Whitney U and Analysis of variance were used to compare differences in mean of variables. The level of statistical significance was determined by a p value of <0.05.

3. RESULTS

Fifty two burns patients were recruited during the study period, but due to attrition (death within 24 hours, patient transfer, industrial actions etc.) only 37 patients had complete data and were thus considered in the analysis (age range; 16-79 years, %TBSA; 20-92%). Fifteen healthy volunteers (age range; 16-55 years) were used for comparison.

The age of the participants in the three groups were comparable; *P*=0.667, while the % TBSA in the two groups of burnt patients were comparable; *P*=0.809. (See Table.1)

Table2. indicates that the mean MDA level of both groups of burns patients at presentation, prior to treatment were similar; *P*=0.272. In like manner the mean TAC level of both groups of burns patients at presentation were similar; *P*=0.165.There was no statistically significant difference in the post-treatment MDA (*P*=0.632) and TAC (*P*=0.675) in the two groups of burns patients.

Table 1. Comparison of participants' characteristics

Variable	Group 1 (Treatment) (n=20)	Group 2 (Placebo) (n-17)	Group 3 (Healthy) (n=15)	Test statistics	p value
Age in years					
Mean±(SD)	35.5±15.8	36.9±13.9	32.6±10.1	0.408*	0.667
Gender					
Male	8 (40.0)	11 (64.7)	13 (86.7)	7.994 †	0.018
Female	12 (60.0)	6 (35.3)	2 (13.3)		
% Total Body Surface Area					
Mean±(SD)	45.5±19.6	47.2±23.5	Not applicable	0.244 ‡	0.809

**: F test †: Chi square test ‡: Student t test*

Table 2. Burns patients' parameters, before and after treatment

Variable	Group 1 (Treatment) (n=20)	Group 2 (Placebo) (n=17)	Student t	p value
Malondialdehyde				
MDA1(nmol/mL)				
Minimum	1.6	1.3		
Maximum	5.9	5.8		
Mean±(SD)	3.5±1.1	3.0±1.3	1.117	0.272
MDA1 OD	0.3±0.1	0.2±0.1	1.079	0.288
MDA 2(nmol/mL)				
Minimum	1.0	1.0		
Maximum	5.0	5.0		
Mean±(SD)	2.6±1.0	2.7±1.1	0.484	0.632
MDA 2 OD	0.2±0.1	0.2±0.1	0.480	0.634
Total Antioxidant Capacity				
TAC 1(µmol/L)				
Minimum	536.8	511.4		
Maximum	987.7	1169.9		
Mean±(SD)	725.6±115.2	797.4 ±177.9	1.477	0.165
TAC 1OD	0.4±0.1	0.5±0.1	1.443	0.174
TAC 2 (µmol/L)				
Minimum	638.2	648.0		
Maximum	1066.5	1404.6		
Mean±(SD)	887.4±117.9	855.8±189.1	0.424	0.675
TAC 2 OD	0.5±0.1	0.5±0.1	0.422	0.676

MDA 1: Pre-treatment malondialdehyde; TAC 1: Pretreatment total anti-oxidant capacity; MDA2: Post-treatment malondialdehyde; TAC 2: Post-treatment total anti-oxidant capacity; OD: Optical density

Table 3 shows that the mean decrease in serum MDA in the burnt patients treated with high dose vitamin C; 0.9±0.8 nmol/mL, was greater than that in those treated with placebo; 0.3±1.4 nmol/mL, but the difference in mean was not statistically significant (*P*=0.106). Similarly the mean increase in TAC in the burnt patients treated with vitamin C; 151.7±116.5 µmol/L was greater than that in those treated with placebo; 58.4±219.1 µmol/L, but the difference in mean was not statistically significant(*P*=0.107).

Table 4, revealed that at presentation, the burns patients had significantly lower TAC than that of the healthy volunteers; (F=5.618, *P*=0.006), while their serum MDA were significantly higher compared to the healthy volunteers (F=3.438, *P*=0.040).

There was no significant correlation between the participant's age or %TBSA and serum MDA and TAC levels (see Table 5).

Fig. 1 depicts the elevated MDA in both groups of burns patients (Groups 1 and 2) compared to healthy volunteers (Group3).

Fig. 2 depicts the decreased TAC in the burns patients (Groups 1 and 2) compared to the healthy volunteers (Group3).

There was no impairment of renal function in any of the burnt patients in either of the groups, as adjudged by the serum creatinine and urea levels. This parameter was considered in all the burnt patients at presentation, and after the treatment interventions.

4. DISCUSSION

In our study, the mean serum malondiadehyde level of the burns patients at presentation was significantly elevated above that of the healthy subjects, while the total antioxidant capacity of burns patients was significantly lower when compared to the healthy subjects. Treatment of the burns patients with 6g of intravenous ascorbic acid over 24 hours was associated with reduced malondialdehyde, and increased total antioxidant capacity; but neither of these effects was statistically significant.

The elevated serum MDA in both groups of burns patients at presentation, compared to healthy volunteers (Table 4.) is in tandem with the earlier finding of Pintaudi and co-workers, who in their evaluation of lipid peroxidation products following acute burns observed increased plasma levels of MDA at baseline in all burns patients, according to the extent of the injury [17]. Our study however revealed no significant correlation between the % TBSA and serum MDA or TAC of the burns patients at presentation (Table 5). Similarly, a Spanish study on severely burnt patients by Farriol et al. found no correlation between TAC and %TBSA [18]. This may suggest that a more comprehensive

Table 3. Effect of treatment on the MDA and TAC serum levels of the groups of burns patients

Variable	Group 1 (Treatment) (n=20)	Group 2 (Placebo) (n=17)	MWU§ p value
Change in MDA (nmol/mL) (Decrease)			
Mean ±(SD)	0.9±0.8	0.3±1.4	MWU 0.106
Median	0.9	0.8	
Change in TAC(µmol/L) (Increase)			
Mean ±(SD)	151.7±116.5	58.4±219.1	MWU 0.107

§: Man Whitney U test

Table 4. Comparison of pre-treatment serum MDA (nmol/mL) and TAC (µmol/L) levels of healthy and burnt participants

Group	n	TAC 1 (Mean ±SD)	F	p value
Treatment Group 1	n=20	725.6±115.2	5.618	0.006
Placebo Group 2	n=17	797.4 ±177.9		
Healthy Group 3	n=15	881.8±104.7		
		MDA 1 (Mean ±SD)		
Treatment Group 1	n=20	3.5±1.1	3.438	0.040
Placebo Group 2	n=17	3.0±1.3		
Healthy Group 3	n=15	2.5±0.7		

Table 5. Correlation between participants' characteristics (Age and TBSA) with pre-treatment MDA and TAC levels

Variable	n	r	p value
Group 1 (Treatment)			
Correlation of MDA 1 with			
Age of respondents in years	n=20	-0.147	0.538
% Total body surface area	(n=20)	0.084	0.724
Group 2 (Placebo)			
Correlation of MDA 1 with			
Age of respondents in years	(n=17)	0.190	0.465
% Total body surface area	(n=17)	-0.288	0.263
Group 3 (Healthy)			
Correlation of MDA 1 with			
Age of respondents	(n=15)	-0.083	0.769
Group 1 (Treatment)			
Correlation of TAC 1 with			
Age of respondents in years	n=20	0.290	0.214
% Total body surface area	(n=20)	0.216	0.359
Group 2 (Placebo)			
Correlation of TAC 1 with			
Age of respondents in years	(n=17)	0.178	0.494
% Total body surface area	(n=17)	0.085	0.745
Group 3 (Healthy)			
Correlation of TAC 1 with			
Age of respondents in years	(n=15)	-0.112	0.690

MDA 1: Pre-treatment malondialdehyde; TAC 1: Pretreatment total anti-oxidant capacity

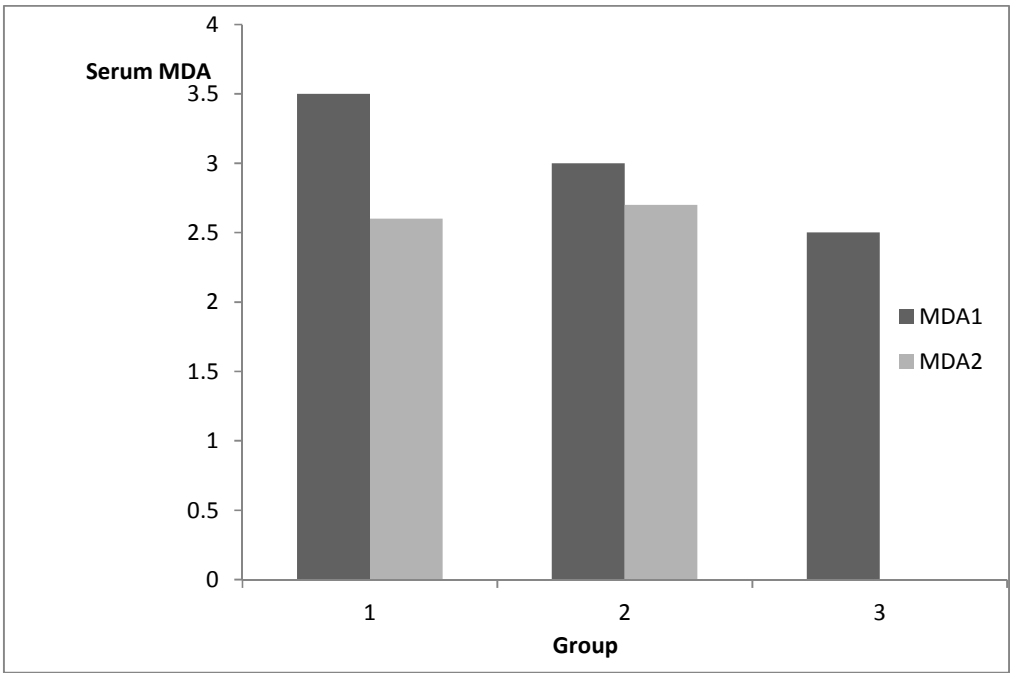


Fig. 1. Bar chart showing patterns of serum MDA (nmol/mL) in the groups of participants

MDA 1: Pre-treatment serum malondialdehyde; MDA 2: Post-treatment serum malondialdehyde

index encompassing other parameters of burns trauma such as thickness of the burn may be a better indicator of severity of oxidative stress. Similarly we found no correlation between the

participants' age and serum MDA or TAC, contrary to the report of Mutlu-Türkoğlu et al. which revealed that age-related oxidative stress was correlated positively with plasma MDA and negatively with TAC [19]. However, although the higher oxidative stress associated with aging would tend to lend greater support to the latter finding, several works have turned in contradictory reports relating to age and indicators of oxidative stress [20].

Among the studies that evaluated TAC in burns patients, Frisman et al. found that TAC was significantly lower in the patients with burn trauma as compared with healthy volunteers [21]. This supports our finding of lower TAC in the burns patients at presentation compared to healthy volunteers (Table4.). Similarly, low TAC levels were observed in another study that compared plasma TAC in children having varying degrees of burns, with healthy children [13].

Al-Jawad et al. [22] measured the serum MDA of burnt patients within 24hr post-burn and recorded elevated levels;5.54±0.34 nmol/L, 6.1±1.1 nmol/L, 5.95 ±1.5 nmol/L, 5.7 ±0.9 nmol/L, 4.86 ±0.7 nmol/L, 6.2±1.1 nmol/L respectively, for the groups that were to receive different antioxidant interventions. Qin et al. [10] also recorded markedly elevated serum MDA levels of 8.97±0.26 nmol/mL, 8.79±0.5 nmol/mL and 8.63±0.54 nmol/mL respectively for the three groups in their series of 36 burns patients prior to receiving various resuscitation modalities. They all presented within 6 hours post-burn. The higher serum MDA levels in these other studies may in part, be a reflection of the temporal course of serum MDA in burns patients as revealed in the earlier studies by Pintaudi et al. [17], Tanaka et al. [6], and Atik et al. [23]. The presentation of the burns patients in the two studies was very early, while most of the patients in our series presented on the second day post-burn.

The mean serum MDA in our healthy volunteers was 2.5±0.7 nmol/mL. Several studies have evaluated serum MDA in healthy subjects and diseased populations [24,25,22]. While there are significant elevations in serum MDA in diseased states, the level of serum MDA quoted in various studies for healthy volunteers are as varied as; 1.66±0.69 nmol/mL [24], 5.32±1.51 nmol/mL [25], 0.81±0.16 nmol/mL [22], and 3.13±0.41 nmol/mL [10]. Several analytical factors may have been responsible for this as different methods were used in the respective analyses. We used the method of Gutteridge and Wilkins [15], to determine the serum MDA in this study.

Whereas Bir et al. [24] used the procedure of Ohkawa et al. [26] to determine serum MDA, Begenik et al. [25] applied the procedure described by Yoshioka et al. [27],

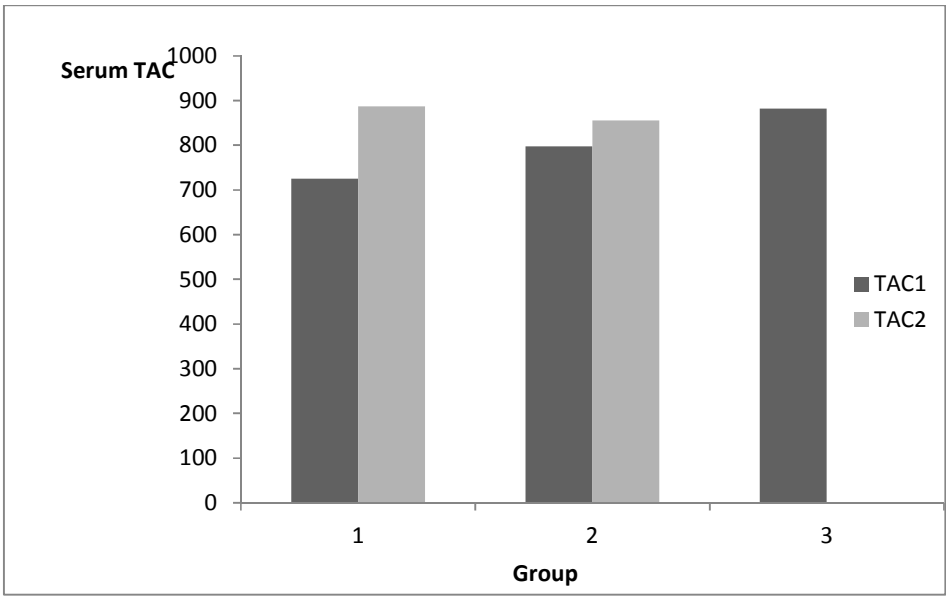


Fig. 2. Bar chart showing patterns of serum TAC (µmol/L) in the groups of participants
TAC 1: Pre-treatment total anti-oxidant capacity; TAC 2: Post-treatment total anti-oxidant capacity

while Al-Jawad et al. [22] measured MDA according to the method described by Stocks and Dormandy [28], as modified by Gilbert et al. [29] Yet still, Qin et al. used the thiobarbituric acid MDA kit for MDA estimation in their recent investigation [10].

Each of the different methods used for TAC estimation measures different TAC components and excludes others. Accordingly, the TAC values obtained in different studies must be compared and interpreted with circumspection since TAC results can be markedly different depending on the assay performed [30,31]. Suresh et al. used the FRAP method to estimate TAC in a population of Indian healthy adults and reported a mean TAC 1018.7 ± 125.6 µmol/L [32]. Another study on healthy Chinese adults had reported a mean TAC of 1017± 206 µmol/L with the FRAP method of TAC estimation [16]. The mean FRAP TAC obtained from our healthy controls was somewhat lower than these; 881.8±104.7 µmol/L, but higher than the FRAP TAC obtained among healthy premenopausal Iranian women; 846.04 ± 152.56 µmol/L [33]. Of note however, was that our screening for health status applied in the recruitment of the control group was essentially limited to exclusion based on information obtained from history taking as indicated in the methodology section. We did not implement anthropometric measurement (for obesity), or biochemical screening (for other pathological conditions such as infections, chronic inflammation, hyperlipidemia, diabetes, liver dysfunction and prostatic hyperplasia). These conditions which may have prevailed in some of our "healthy group" are known to impose considerable oxidative stress and may have impacted on our results by lowering the TAC of our "healthy group". Furthermore the higher TAC obtained from the Indian and Chinese populations may have been influenced by dietary and lifestyle differences, both being factors that have also been proven to affect TAC.

At the dose of 6g intravenous vitamin C over 24 hours which we used in our treatment group we noted reduced serum MDA, and increased TAC; but neither of these effects reached statistical significance. We informed earlier that as part of routine care in our hospital setting where this study was conducted, all the burns patients (both treatment and placebo groups) received Ascorbic acid tablets (1g/day), vitamin E capsules (1000 iu/day) and vitamin A capsules (10,000 iu/day) as part of routine care, starting from the day of presentation. In effect the burns patients in both the treatment and placebo groups received substantial amount of anti-oxidants, but with the treatment group receiving a comparatively higher dose of antioxidants owing to the extra high-dose vitamin C infusion (6 g). Thus the potential effect of this in blurring the real impact of the intravenous vitamin C therapy in the "treatment group" relative to the "placebo group" can only be imagined. In the study by Qin et al. [10] the vitamin C treatment group received vitamin C infusion at the dose of 33 mg/kg/hr for 7 days plus 'normal care' while the control group received only 'normal care'. At 24 hrs after commencement of treatment the vitamin C group in their study had lower serum MDA level compared to normal care group but that was not statistically significant, however at 48 hrs the greater reduction of serum MDA in the vitamin C group was significantly different; $p < 0.05$. Their dose of vitamin C despite being much lower than the 66mg/kg/hr administered to burns patients by Tanaka et al. [6] was efficacious in attenuating oxidative stress in burns patients.

With the few case reports of oxalate nephropathy and acute kidney injury in burns patients who received excess of 100 g vitamin C within 24 hours based on the dose of 66 mg/kg/hr, there is concern for its safety [9]. Hence the need to explore for the minimum dose of mega-dose vitamin C, with both satisfactory efficacy and safety for burns patients. We observed no impairment of renal function in any of the burnt patients, as adjudged by the serum creatinine and urea levels, measured before and after the therapeutic interventions. Earlier studies that utilized much higher doses of intravenous vitamin C; 66 mg/kg/hr [6,8] and 33 mg/kg/hr [10] had also reported absence of signs of renal insufficiency at such high doses.

There is evidence that oxidative injury following burns continues for several weeks [17]. There have been few studies conducted with high-dose ascorbic acid in burns patients, but this is an area of evolving interest. Evidence from few published works however suggest that the effect of high-dose antioxidants in suppressing oxidative stress is most pronounced in the early period after the burns. Owing to safety concerns regarding potential renal toxicity with prolonged high-dose ascorbic acid, the duration of the therapy has been restricted in most studies. The absence of renal impairment with the dose of ascorbic acid used in this study is reassuring, and will encourage the use of higher doses for longer periods, in order to achieve optimal effect.

5. LIMITATIONS AND RECOMMENDATIONS

Owing to our lack of bed weighing scale or pit-mounted scale to facilitate the weighing of the ill burnt patient (who couldn't be reasonably expected to stand on a regular floor scale), we were constrained to use a universal dose of 6g for all the adult burns patients, irrespective of their obvious weight differences. As earlier stated, the high dose of antioxidants which were administered concurrently to all the burns patients (both treatment and placebo groups) as part of routine care could have greatly masked the distinctive effect of the intravenous vitamin C therapy in the comparison with those treated with placebo. Nevertheless, we recommend exploring a vitamin C dose higher than 6g/24hour in future studies in view of the observed trend in reducing MDA and increasing TAC in our study, albeit short of statistically significant degree.

6. CONCLUSION

Burns injury impacted quantifiable oxidative stress as measured by elevated MDA and decreased TAC in the burns patients compared to the healthy controls. However, the decrease in MDA and increase in TAC achieved in the patients resuscitated with adjuvant intravenous vitamin C at a dose of 6g over 24 hrs was not statistically significant. This dose of vitamin C was not associated with any obvious renal derangement based on the biochemical evaluation of the burnt patients' serum urea and creatinine levels.

CONSENT AND ETHICAL APPROVAL

The research ethics committee of National Orthopaedic Hospital, Enugu gave approval for the study [IRB/HEC/ Number S. 313/IV; Protocol number 106]. Informed consent was sought and obtained from each participant before recruitment into the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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