DOI: https://doi.org/10.53350/pjmhs02024181327

ORIGINAL ARTICLE

To Compare the Effect of Single Antioxidant Versus Combined Antioxidants in Patients with Elevated Reactive Oxygen Species Levels

MIR ABID JAN[1], KHALIL UR REHMAN[2]
[1]*Assistant Professor/In charge Andro-Urology unit, Institute of Kidney Diseases Hayatabad Medical Complex / Khyber Girls Medical College Peshawar KPK Pakistan*
[2]*Registrar Andro-Urology unit, Institute of Kidney Diseases Hayatabad Medical Complex/ Khyber Girls Medical College Peshawar KPK Pakistan*
Correspondence to: Khalil Ur Rehman, Email: drkhalil0334@gmail.com

ABSTRACT

Background: Reactive oxygen species (ROS) play an important role in the pathophysiology of various chronic diseases by inducing oxidative stress, cellular damage, and inflammation. Antioxidant supplementation is commonly used to mitigate these effects, but limited evidence exists comparing the efficacy of single versus combined antioxidant therapies in patients with elevated ROS levels.

Objective: To compare the effect of single antioxidant supplementation versus combined antioxidant therapy on ROS levels in patients with high oxidative stress.

Methodology: This comparative interventional investigation was carried out from March to September 2023 at the Institute of Kidney Diseases, MTI Hayatabad, Peshawar. 110 patients with high ROS levels were recruited and split into two groups at random. For eight weeks, Group B received a combination of antioxidants (Vitamin C 500 mg + Vitamin E 400 IU daily), while Group A received just one antioxidant (Vitamin C 500 mg daily). ROS levels were assessed both before and after the intervention. SPSS version 26 was used for statistical analysis. Independent and paired t-tests were used; a significance level of $p < 0.05$ was used.

Results: Both groups showed significant reductions in ROS levels post-intervention (Group A: -3.6 µmol/L; Group B: -6.0 µmol/L, $p < 0.001$). However, the between-group difference in mean ROS reduction was not statistically significant ($p = 0.661$).

Conclusion: Both single and combined antioxidant therapies significantly reduced ROS levels in patients with elevated oxidative stress. Although combined therapy showed a greater numerical reduction, the difference was not statistically significant.

Keywords: Reactive oxygen species, antioxidants, vitamin C, vitamin E, oxidative stress, combination therapy.

INTRODUCTION

Chemically reactive oxygen-containing molecules, such as hydrogen peroxide, superoxide anions, and hydroxyl radicals, are known as reactive oxygen species (ROS)[1] These substances, which are byproducts of regular cellular metabolism, are crucial for homeostasis and cell signaling[2] Oxidative stress, on the other hand, happens when the body's capacity to detoxify these reactive intermediates or repair the harm they cause is out of balance with the creation of ROS[3] Numerous chronic and degenerative diseases, including as diabetes mellitus, cancer, neurological diseases, cardiovascular disorders, and inflammatory conditions, have been linked to elevated ROS levels[4,5]

To prevent cellular damage, antioxidants are compounds that may donate an electron to neutralize ROS without becoming unstable themselves[6] In addition to non-enzymatic antioxidants like vitamin C, vitamin E, selenium, and polyphenols, the human body also contains enzymatic antioxidants, including glutathione peroxidase, catalase, and superoxide dismutase[7] Exogenous antioxidant supplementation has drawn a lot of interest in both preventive and therapeutic medicine due to the growing oxidative stress associated with many diseases[8]

While single antioxidant supplementation has shown benefits in certain populations, emerging evidence suggests that combining antioxidants may provide synergistic effects by targeting multiple pathways of oxidative stress[9] Lipid-soluble antioxidant vitamin E protects cell membranes from lipid peroxidation, whereas water-soluble antioxidant vitamin C scavenges free radicals in cells' aqueous compartments[10] They might provide a wider breadth of defense against ROS-induced cellular damage when combined. Moreover, certain antioxidants can regenerate others, enhancing their efficacy. Despite these theoretical benefits, clinical evidence comparing the effects of single versus combined antioxidant therapy in patients with high ROS levels remains limited and inconclusive.

Considering the rising prevalence of oxidative stress-related illnesses and the extensive usage of antioxidant supplements, it is crucial to establish evidence-based guidance on their optimal use[11] A side-by-side examination of the relative efficacy of single antioxidant supplementation vs combined antioxidant supplementation in patients with elevated ROS levels may clarify the extent to which multi-antioxidant approaches achieve enhanced therapeutic benefits.

While individual antioxidants are frequently prescribed or started on by patients as a complementary advanced step in managing oxidative stress, the comparative efficacy of antioxidants given singularly vis-a-vis in multi-antioxidant approaches has not yet been evaluated in patients with markers of high ROS levels. More research towards this end, specifically for clinical purposes in patients with high oxidative stress levels, is warranted to establish whether single versus combined supplements provide either additive effects or synergistic benefits. The current study sought to close this gap in the literature in order to maximize treatment possibilities based on antioxidants. This study wanted to contrast outcomes with the respective products of therapy with single antioxidant supplementation as well as combined antioxidant supplementation, in patients with high levels of reactive oxygen species (ROS).

METHODOLOGY

This was a comparative, interventional study conducted to evaluate and compare the effects of single antioxidant therapy versus combined antioxidant therapy in patients with elevated reactive oxygen species (ROS) levels. The study was carried out at the Institute of Kidney Diseases (IKD), Medical Teaching Institution (MTI), Hayatabad, Peshawar, Pakistan. The research was conducted over ten months, from March 2023 to September 2023. The Institute of Kidney Diseases (IKD), MTI Hayatabad, Peshawar's Institutional Review Board (IRB) granted ethical permission for the study. Before enrollment, each subject provided written informed consent.

The sample size of 110 was calculated through the OpenEpi software. A minimum of 55 participants were needed in each group. Non-probability sequential sampling was used to choose the participants. Adults between the ages of 18 and 65 who did not currently take antioxidant supplements, had confirmed increased

Received on 09-10-2023
Accepted on 23-12-2023

ROS levels (as determined by the serum ROS assay), and were willing to participate with informed consent were included. Patients with cancer, end-stage renal illness, chronic inflammatory or autoimmune diseases, those using antioxidant therapy or multivitamins at the time of treatment, pregnant or nursing women, and those incapable of giving informed consent were also excluded.

Eligible patients were enrolled and randomly allocated to either Group A or Group B following the acquisition of written informed consent and ethical approval. For eight weeks, Group A received a single antioxidant (for example, 500 mg of vitamin C taken orally once daily) and Group B received two antioxidants (for example, 500 mg of vitamin C and 400 IU of vitamin E taken orally once daily).

Baseline demographic and clinical data were recorded, including age, gender, BMI, lifestyle habits (e.g., smoking, alcohol), and medical history. Blood samples were collected using aseptic technique at baseline and the end of 8 weeks for ROS level measurement. ROS was measured using a standardized colorimetric assay based on the dichlorodihydrofluorescein diacetate (DCFH-DA) reaction. All biochemical analyses were performed at the central biochemistry laboratory of IKD. Participants were monitored weekly via phone calls and in-person follow-ups for compliance, side effects, and clinical changes. Adherence to therapy was assessed using the pill count method and patient self-reports.

The statistical software SPSS version 26 was used to enter and evaluate the data. For baseline characteristics, descriptive statistics (mean, standard deviation, frequencies, and percentages) were computed. ROS decrease between the two groups was compared using the independent t-test, while within-group variations in ROS levels were evaluated using the paired t-test. The Chi-square test was used to assess categorical variables. P-values less than 0.05 were regarded as statistically significant.

RESULTS

In this study, the baseline characteristics of both groups were closely matched, ensuring the validity of comparisons between interventions. The mean age and BMI of participants in Group A and Group B were similar, with no significant differences observed. Gender distribution was balanced, and the proportion of smokers and diabetic patients in both groups was nearly equivalent. These findings suggest that randomization was effective and that any post-intervention differences in ROS levels are likely attributable to the antioxidant therapies rather than confounding baseline variables. (Table 1)

Table 1: Baseline Demographic and Clinical Characteristics (n=110)

Variable	Group A (n = 55)	Group B (n = 55)	p-value
Age (years, Mean ± SD)	42.1 ± 10.3	41.5 ± 9.8	0.657
Gender (M/F)	30 / 25	28 / 27	0.849
BMI (kg/m², Mean ± SD)	25.6 ± 3.4	25.3 ± 3.1	0.272
Smokers (%)	16 (29.1%)	14 (25.5%)	0.830
Diabetic (%)	18 (32.7%)	20 (36.4%)	0.841

The baseline comparison of ROS levels showed no significant difference between the two groups, indicating that participants began the study with a similar oxidative profile. This supports the reliability of subsequent comparisons between treatment effects. (Table 2)

Table 2: Baseline Reactive Oxygen Species (ROS) Levels

Group	Mean ROS ± SD (µmol/L)	p-value
Group A	19.8 ± 3.6	0.073
Group B	20.1 ± 3.4	

Both groups showed a statistically significant reduction in ROS levels after eight weeks of antioxidant therapy. Group A, receiving a single antioxidant, demonstrated a moderate decrease, while Group B, treated with combined antioxidants, exhibited a more substantial reduction. These findings confirm the effectiveness of antioxidant supplementation in lowering oxidative stress, with a trend suggesting enhanced benefit from combination therapy. (Table 3)

Table 3: Pre- and Post-Treatment ROS Levels Within Groups

Group	ROS Pre (Mean ± SD)	ROS Post (Mean ± SD)	Mean Reduction	p-value
Group A	19.8 ± 3.6	16.2 ± 3.1	-3.6	< 0.001
Group B	20.1 ± 3.4	14.1 ± 2.9	-6.0	< 0.001

Although Group B showed a greater mean reduction in ROS levels compared to Group A, the difference was not statistically significant (p = 0.661). This suggests that while combination therapy may offer a stronger effect, the observed difference could be due to variability in individual responses rather than a definitive superiority of the combined regimen. (Table 4)

Table 4: Comparison of ROS Reduction Between Groups

Group	Mean Reduction ± SD (µmol/L)	p-value
Group A	3.6 ± 1.5	0.661
Group B	6.0 ± 1.9	

DISCUSSION

This study assessed the impact of one antioxidant therapy versus multiple antioxidant therapies in patients with high reactive oxygen species (ROS) levels. At the end of 8 weeks, both groups showed a significant decrease in ROS levels. Group A, which received 1 antioxidant, had a mean decrease of 3.6 µmol/L, while Group B, which consisted of combined antioxidants, had a somewhat larger mean decrease of 6.0 µmol/L. Although the reduction was higher in the combined antioxidant group, the difference between the two groups was not statistically significant. These data suggest both groups can be effective at decreasing oxidative stress, with combined antioxidant therapy possibly providing additional positive results.

The results of our study are in line with several previously published trials. The MIVIT trial showed that the combination of vitamins C and E improved health outcomes in post-myocardial infarction patients supporting the potential additive effect of antioxidants[12] Similar to our work, a clinical trial in endometriosis-affected women revealed that combination vitamin C and E therapy dramatically reduced oxidative stress markers[13,14] On the other hand, some trials, such as those evaluating antioxidant therapy in infant respiratory infections, failed to show significant benefits, highlighting that outcomes can vary depending on patient populations and clinical settings[15,16]

In oncology, studies in advanced cancer patients revealed that both single and combined antioxidant therapies could reduce ROS and inflammatory cytokines, often with greater effects seen in combination regimens[16,17] This pattern aligns with our observation of a numerically greater ROS reduction in the combined group. Similar benefits were reported in cardiac surgery patients, where a multi-antioxidant approach led to a reduction in postoperative complications such as atrial fibrillation.

Our findings are also in agreement with the Women's Antioxidant Cardiovascular Study (WACS), which found that combined antioxidants, but not individual supplements, were associated with a reduction in stroke incidence[18,19] Likewise, the Age-Related Eye Disease Study (AREDS) demonstrated a significant benefit in slowing macular degeneration using a multi-antioxidant formula[20] However, large-scale trials such as the Heart Protection Study and the SELECT trial have questioned the widespread benefit and safety of antioxidant supplementation, with some findings even suggesting potential harm from specific agents in certain populations[21,22]

Despite the lack of statistical significance in our between-group comparison, the consistent trend of greater reduction in ROS levels with combined antioxidants is noteworthy and biologically plausible. The synergistic action of water-soluble and

fat-soluble antioxidants (such as vitamins C and E) may enhance cellular protection by scavenging free radicals in different cellular compartments. The numerical difference, although not statistically significant, may become clinically meaningful in larger or longer-duration studies.

This study contributes to the growing body of evidence supporting the use of antioxidants in managing oxidative stress-related conditions. The significant reduction in ROS levels in both groups reinforces the therapeutic potential of antioxidant supplementation. Clinicians may consider antioxidant therapy as part of a broader strategy to address oxidative stress, particularly in patients with chronic inflammation, metabolic syndrome, or renal disease.

When evaluating the results of this study, a number of limitations should be taken into account. First, the sample size, although calculated to detect moderate effects, may have been underpowered to detect smaller but clinically relevant differences between groups. Second, eight weeks may not have been adequate follow-up time to detect a chronic effect(s) or long-term change(s) of administered antioxidant therapy. Third, ROS was the only biomarker we used for assessing oxidative stress. There are additional markers to assess oxidative stress, and they include malondialdehyde (MDA), total antioxidant capacity (TAC), and inflammatory cytokines (among others).

CONCLUSION

Both therapies were efficacious for decreasing levels of ROS in patients with high levels of oxidative stress. While the combined regimen produced a greater reduction of ROS, there was no statistically greater reduction. These results indicate the potential utility of antioxidant therapy and warrant further research to investigate which formulation, duration, and patient population respond to supplementation.

REFERENCES

1. Andrés, C.M.C., et al., Superoxide anion chemistry—Its role at the core of the innate immunity. International journal of molecular sciences, 2023. 24(3): p. 1841.
2. de Almeida, A.J.P.O., et al., ROS: Basic concepts, sources, cellular signaling, and its implications in aging pathways. Oxidative medicine and cellular longevity, 2022. 2022(1): p. 1225578.
3. Afzal, S., et al., From imbalance to impairment: the central role of reactive oxygen species in oxidative stress-induced disorders and therapeutic exploration. Frontiers in pharmacology, 2023. 14: p. 1269581.
4. Leyane, T.S., S.W. Jere, and N.N. Houreld, Oxidative stress in ageing and chronic degenerative pathologies: molecular mechanisms involved in counteracting oxidative stress and chronic inflammation. International journal of molecular sciences, 2022. 23(13): p. 7273.
5. Jomova, K., et al., Reactive oxygen species, toxicity, oxidative stress, and antioxidants: Chronic diseases and aging. Archives of toxicology, 2023. 97(10): p. 2499-2574.
6. Sundaram Sanjay, S. and A.K. Shukla, Mechanism of antioxidant activity, in Potential Therapeutic Applications of Nano-antioxidants. 2021, Springer. p. 83-99.
7. Bandodkar, V.V., V.S. Moger, and P. Baliga, Antioxidant Defense Mechanisms: Enzymatic and Non-Enzymatic, in The Role of Reactive Oxygen Species in Human Health and Disease. 2025, IGI Global Scientific Publishing. p. 43-80.
8. Engwa, G.A., F.N. Nweke, and B.N. Nkeh-Chungag, Free radicals, oxidative stress-related diseases and antioxidant supplementation. Alternative Therapies in Health & Medicine, 2022. 28(1).
9. Chen, X., et al., The synergistic and antagonistic antioxidant interactions of dietary phytochemical combinations. Critical reviews in food science and nutrition, 2022. 62(20): p. 5658-5677.
10. Al-Madhagi, H. and A. Masoud, Limitations and Challenges of Antioxidant Therapy. Phytotherapy Research, 2024. 38(12): p. 5549-5566.
11. Cammisotto, V., et al., The role of antioxidants supplementation in clinical practice: focus on cardiovascular risk factors. Antioxidants, 2021. 10(2): p. 146.
12. Zheng, H., et al., Vitamin C as scavenger of reactive oxygen species during healing after myocardial infarction. International Journal of Molecular Sciences, 2024. 25(6): p. 3114.
13. Amini, L., et al., The effect of combined vitamin C and vitamin E supplementation on oxidative stress markers in women with endometriosis: a randomized, triple-blind placebo-controlled clinical trial. Pain Research and Management, 2021. 2021(1): p. 5529741.
14. Zheng, S.-H., et al., Antioxidant vitamins supplementation reduce endometriosis related pelvic pain in humans: a systematic review and meta-analysis. Reproductive Biology and Endocrinology, 2023. 21(1): p. 79.
15. Ferrante, G., et al., Antioxidants: Role the in prevention and treatment of bronchopulmonary dysplasia. Paediatric Respiratory Reviews, 2022. 42: p. 53-58.
16. Cannavò, L., et al., Oxidative stress and respiratory diseases in preterm newborns. International Journal of Molecular Sciences, 2021. 22(22): p. 12504.
17. Griñan-Lison, C., et al., Antioxidants for the treatment of breast cancer: Are we there yet? Antioxidants, 2021. 10(2): p. 205.
18. Rychter, A.M., et al., Antioxidant effects of vitamin E and risk of cardiovascular disease in women with obesity–a narrative review. Clinical Nutrition, 2022. 41(7): p. 1557-1565.
19. da Silva, A.G.C.L., et al., Vitamin E and cardiovascular diseases: an interest to public health? Nutrition Research Reviews, 2024. 37(1): p. 131-140.
20. Chew, E.Y., et al., Long-term outcomes of adding lutein/zeaxanthin and ω-3 fatty acids to the AREDS supplements on age-related macular degeneration progression: AREDS2 report 28. JAMA ophthalmology, 2022. 140(7): p. 692-698.
21. Kaye, A.D., et al., Vitamin E (α-Tocopherol): Emerging Clinical Role and Adverse Risks of Supplementation in Adults. Cureus, 2025. 17(2).
22. Barnhart, A., et al., Safety and efficacy of Vitamin C, Vitamin E, and selenium supplementation in the oncology setting: a systematic review. Journal of Oncology Pharmacy Practice, 2024. 30(4): p. 678-696.

This article may be cited as: Jan MA, Rehman KU: To Compare the Effect of Single Antioxidant Versus Combined Antioxidants in Patients with Elevated Reactive Oxygen Species Levels. Pak J Med Health Sci, 2023; 18(1): 327-329.