International Physiology

(A PEER-REVIEWED AND REFEREED JOURNAL)

VOLUME 12 NUMBER 2-3, MAY - DECEMBER 2024

RED FLOWER PUBLICATIONS PVT LTD
New Delhi - 110091

Revised Rates for 2025 (Institutional)

Title of the Journal	Frequency	India (INR) Print Only	India (INR) Online Only	Outside India (USD) Print Only	Outside India (USD) Online Only
Community and Public Health Nursing	Triannual	7,000	6,500	$547	$508
Indian Journal of Agriculture Business	Semiannual	7,000	6,500	$547	$508
Indian Journal of Anatomy	Triannual	10,000	9,500	$781	$742
Indian Journal of Ancient Medicine and Yoga	Quarterly	9,500	9,000	$742	$703
Indian Journal of Anesthesia and Analgesia	Quarterly	9,000	8,500	$703	$664
Indian Journal of Biology	Semiannual	7,000	6,500	$547	$508
Indian Journal of Cancer Education and Research	Semiannual	10,500	10,000	$820	$781
Indian Journal of Communicable Diseases	Semiannual	10,000	9,500	$781	$742
Indian Journal of Dental Education	Triannual	7,000	6,500	$547	$508
Indian Journal of Diabetes and Endocrinology	Semiannual	9,500	9,000	$742	$703
Indian Journal of Emergency Medicine	Quarterly	14,000	13,500	$1094	$1055
Indian Journal of Forensic Medicine and Pathology	Quarterly	17,500	17,000	$1367	$1328
Indian Journal of Forensic Odontology	Semiannual	7,000	6,500	$547	$508
Indian Journal of Genetics and Molecular Research	Semiannual	8,500	8,000	$664	$625
Indian Journal of Law and Human Behavior	Semiannual	7,500	7,000	$586	$547
Indian Journal of Legal Medicine	Semiannual	10,000	9,500	$781	$742
Indian Journal of Library and Information Science	Triannual	11,000	10,500	$859	$820
Indian Journal of Maternal-Fetal & Neonatal Medicine	Semiannual	11,000	10,500	$859	$820
Indian Journal of Medical and Health Sciences	Semiannual	8,500	8,000	$664	$625
Indian Journal of Obstetrics and Gynecology	Quarterly	11,000	10,500	$859	$820
Indian Journal of Pathology: Research and Practice	Triannual	13,500	13,000	$1055	$1016
Indian Journal of Plant and Soil	Semiannual	8,000	7,500	$625	$586
Indian Journal of Preventive Medicine	Semiannual	8,500	8,000	$664	$625
Indian Journal of Research in Anthropology	Semiannual	14,000	13,500	$1094	$1055
Indian Journal of Surgical Nursing	Triannual	7,000	6,500	$547	$508
Indian Journal of Trauma and Emergency Pediatrics	Triannual	11,000	10,500	$859	$820
Indian Journal of Waste Management	Semiannual	11,000	10,500	$859	$820
International Journal of Food, Nutrition & Dietetics	Triannual	7,000	6,500	$547	$508
International Journal of Forensic Science	Semiannual	11,500	11,000	$898	$859
International Journal of Neurology and Neurosurgery	Triannual	12,000	11,500	$938	$898
International Journal of Pediatric Nursing	Triannual	7,000	6,500	$547	$508
International Journal of Political Science	Semiannual	7,500	7,000	$586	$547
International Journal of Practical Nursing	Triannual	7,000	6,500	$547	$508
International Physiology	Semiannual	9,000	8,500	$703	$664
Journal of Animal Feed Science and Technology	Semiannual	9,500	9,000	$742	$703
Journal of Cardiovascular Medicine and Surgery	Quarterly	11,500	11,000	$898	$859
Journal of Emergency and Trauma Nursing	Semiannual	7,000	6,500	$547	$508
Journal of Forensic Chemistry and Toxicology	Semiannual	11,000	10,500	$859	$820
Journal of Global Medical Education and Research	Semiannual	7,500	7,000	$586	$547
Journal of Global Public Health	Semiannual	13,500	13,000	$1055	$1016
Journal of Microbiology and Related Research	Semiannual	10,000	9,500	$781	$742
Journal of Nurse Midwifery and Maternal Health	Triannual	7,000	6,500	$547	$508
Journal of Orthopedic Education	Semiannual	7,000	6,500	$545	$508
Journal of Pharmaceutical and Medicinal Chemistry	Semiannual	18,000	17,500	$1406	$1367
Journal of Plastic Surgery and Transplantation	Semiannual	28,000	27,500	$2188	$2148
Journal of Psychiatric Nursing	Triannual	7,000	6,500	$547	$508
Journal of Radiology	Semiannual	9,500	9,000	$742	$703
Journal of Social Welfare and Management	Quarterly	9,000	8,500	$703	$664
New Indian Journal of Surgery	Quarterly	9,500	9,000	$742	$703
Ophthalmology and Allied Sciences	Semiannual	7,500	7,000	$586	$547
Pediatrics Education and Research	Semiannual	9,000	8,500	$703	$664
Physiotherapy and Occupational Therapy Journal	Quarterly	10,500	10,000	$820	$781
RFP Gastroenterology International	Semiannual	7,500	7,000	$586	$547
RFP Indian Journal of Hospital Infection	Semiannual	14,000	13,500	$1094	$1055
RFP Indian Journal of Medical Psychiatry	Semiannual	9,500	9,000	$742	$703
RFP Journal of Biochemistry and Biophysics	Semiannual	8,500	8,000	$664	$625
RFP Journal of Dermatology	Semiannual	7,000	6,500	$547	$508
RFP Journal of ENT and Allied Sciences	Semiannual	7,000	6,500	$547	$508
RFP Journal of Gerontology and Geriatric Nursing	Semiannual	7,000	6,500	$547	$508
RFP Journal of Hospital Administration	Semiannual	8,500	8,000	$664	$625
Urology, Nephrology and Andrology International	Semiannual	9,000	8,500	$703	$664

Terms of Supply:

1. Agency discount 15% (This discount is applicable only 2025 subscription for agencies only). Issues will be sent directly to the end user, otherwise foreign rates will be charged.
2. All back volumes of all journals are available at current rates.
3. All journals are available free online with print order within the subscription period.
4. All legal disputes subject to Delhi jurisdiction.
5. Cancellations are not accepted orders once processed.
6. Demand draft/cheque should be issued in favour of "**Red Flower Publication Pvt. Ltd.**" payable at **Delhi.**
7. Full pre-payment is required. It can be done through online (http://rfppl.co.in/subscribe.php?mid=7).
8. No claims will be entertained if not reported within 6 months of the publishing date.
9. Orders and payments are to be sent to our office address as given below.
10. Postage & Handling is included in the subscription rates.
11. Subscription period is accepted on calendar year basis (i.e. Jan to Dec). However orders may be placed any time throughout the year.

Order from

Red Flower Publication Pvt. Ltd., 48/41-42, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi - 110 091 (India)
Mobile: 8130750089, Phone: 91-11-79695648 E-mail: sales@rfppl.co.in, Website: www.rfppl.co.in

INTERNATIONAL PHYSIOLOGY

Editor-in-Chief

Rajesh Pathak,
Additional Principal,
Senior Professor and Head of Department Physiology
Jawahar Lal Nehru Medical College & Associated Group of Hospitals,
Ajmer 305001, Rajasthan, India.

Executive Editor

Amit Kant Singh
Uttar Pradesh

International Editorial Board Member

Dale D Tang, Albany Medical College, New York

National Editorial Board Member

Bharati Mehta,
All India Institute of Medical Sciences, Jodhpur

Kiran H Buge,
Dr. Vithalrao Vikhe Patil Foundation's Medical College, Maharashtra

Deepak Kumar Das,
Varun Arjun Medical College & Rohilkhand Hospital, Uttar Pradesh

Anupkumar Dhanvijay,
All India Institute of Medical Sciences, Deoghar, Jharkhand

Pranati Nanda,
All India Institute of Medical Sciences, Bhubaneswar, Odisha

Managing Editor: A Lal
E-mail: info@rfppl.co.in

Publication Editor: Dinesh Kr. Kashyap
E-mail: author@rfppl.co.in

The International Physiology (pISSN: 2347–1506, eISSN: 2455-6262) publishes study of function in these systems, such as biochemistry, immunology, genetics, mathematical modeling, molecular biology, and physiological methodologies. Papers on the basis of pathophysiological diseases such on processes of the kidney, urinary tract, and regulation of body fluids are also encouraged. Papers dealing with topics in other basic sciences that impinge on physiology are also welcome. Moreover, theoretical articles on research at any level of biological organization ranging from molecules to humans fall within the broad scope of the Journal.

For all other queries Red Flower Publication Pvt. Ltd., 48/41-42, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi–110 091 (India), Phone: 91-11-79695648, E-mail: info@rfppl.co.in, Web:www.rfppl.co.in

Disclaimer The opinion in this publication is those of the authors and is not necessarily those of the International Physiology the Editor-in-Chief and Editorial Board. Appearance of an advertisement does not indicate International Physiology approval of the product or service.

© Red Flower Publication Pvt. Ltd. 2024 all rights reserved. No part of the journal may be reproduce, stored in a retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without prior permission of the New Indian Journal of Surgery.

Red Flower Publication (P) Ltd.

Presents its Book Publications for sale

1. **Beyond Medicine: A to E for Medical Professionals) (2020)**
   Kalidas Chavan
   INR390/USD31
2. **Biostatistical Methods For Medical Research (2019)**
   Sanjeev Sarmukaddam **INR549/USD44**
3. **Breast Cancer: Biology, Prevention And Treatment (2015)**
   Dr. A. Ramesh Rao **INR 395/USD31**
4. **Chhotanagpur A Hinterland of Tribes (2020)**
   Ambrish Gautam **INR250/ USD20**
5. **Child Intelligence (2004)**
   Dr. Rajesh Shukla, Md, Dch. **INR100/ USD50**
6. **Clinical Applied Physiology and Solutions (2020)**
   Varun Malhotra **INR263/USD21**
7. **Comprehensive Medical Pharmacology (2019)**
   Dr. Ahmad Najmi **INR599/USD47**
8. **Critical Care Nursing in Emergency Toxicology (2019)**
   Vivekanshu Verma **INR460/USD34**
9. **Digital Payment (Blue Print For Shining India) (2020)**
   Dr. Bishnu Prasad Patro **INR329/USD26**
10. **Drugs in Anesthesia (2020)**
    R. Varaprasad **INR449/USD35**
11. **Drugs In Anesthesia and Critical Care (2020)**
    Dr. Bhavna Gupta **INR595/USD46**
12. **MCQs in Medical Physiology (2019)**
    Dr. Bharati Mehta **INR300/ USD29**
13. **MCQs in Microbiology, Biotechnology and Genetics (2020)**
    Biswajit Batabyal **INR285/USD22**
14. **MCQs In Minimal Access and Bariatric Surgery (2nd Edition) (2020)**
    Anshuman Kaushal **INR545/USD42**
15. **Patient Care Management (2019)**
    A.K. Mohiuddin **INR999/USD78**
16. **Pediatrics Companion (2001)**
    Rajesh Shukla **INR 250/USD50**
17. **Pharmaceutics-1 (A Comprehensive Hand Book) (2021)**
    V. Sandhiya **INR525/ USD50**
18. **Poultry Eggs of India (2020)**
    Prafulla K. Mohanty **INR390/USD30**
19. **Practical Emergency Trauma Toxicology Cases Workbook (2019)**
    Dr. Vivekanshu Verma, Dr. Shiv Rattan Kochar, Dr. Devendra Richhariya **INR395/USD31**
20. **Practical Record Book of Forensic Medicine & Toxicology (2019)**
    Dr. Akhilesh K. Pathak **INR299/USD23**
21. **Recent Advances in Neonatology (2020)**
    Dr. T.M. Ananda Kesavan **INR 845/USD66**
22. **Shipping Economics (2018)**
    Dr. D. Amutha **INR347/USD45**
23. **Skeletal and Structural Organizations of Human Body (2019)**
    Dr. D.R. Singh **INR659/USD51**
24. **Statistics In Genetic Data Analysis (2020)**
    S.Venkatasubramanian **INR299/USD23**
25. **Synopsis of Anesthesia (2019)**
    Dr. Lalit Gupta **INR1195/USD75**
26. **A Handbook of Outline of Plastic Surgery Exit Examination (2022)**
    Prof Ravi Kumar Chittoria & Dr. Saurabh Gupta **INR 498/USD 38**
27. **An Introductory Approach to Human Physiology (2021)**
    Satyajit Tripathy, Barsha Dasssarma, Motlalpula Gibert Matsabisa **INR 599/USD 46**
28. **Biochemical and Pharmacological Variations in Venomous Secretion of Toad (Bufo melanostictus)(2021)**
    Dr. Thirupathi Koila & Dr. Venkaiah Yanamala **INR 325/USD26**
29. **Climate, Prey & Predator Insect Poupulation in Bt Cotton and Non-Bt Cotton Agriculture Feilds of Warangal District (2022)**
    Dr. Peesari Laxman,Ch. Sammaiah **INR 325/USD26**
30. **Community Health Nursing Record Book Volume – I & II (2022)**
    Ritika Rocque **INR 999/USD 79**
31. **Handbook of Forest Terminologies (Volume I & II) (2022)**
    Dr. C.N.Hari Prasath, Dr. A. Balasubramanian, Dr. M. Sivaprakash, V. Manimaran, Dr. G. Swathiga **INR 1325/USD 104**
32. **MCQs of Biochemistry(2022)**
    Sachin C. Narwadiya, Dr. Irfana Begum **INR 399/USD 49**
33. **Newborn Care in the State of Uttar Pradesh(2022)**
    Dr. Tridibesh Tripathy **INR 545/USD 42**
34. **Osteoporosis: Weak Bone Disease(2022)**
    Dr. Dondeti Uday Kumar & Dr. R. B. Uppin **INR 399/USD49**
35. **Quick Updates in Anesthesia(2022)**
    Dr. Rupinder Kaur Kaiche, Dr. Vidhyadhar Modak, Dr. Shilpa Sannakki & Dr. Vivek Gupta **INR 599/USD 44**
36. **Textbook of Practice of Medicine with Homoeopathic Therapeutics(2022)**
    Dr. Pramod Kumar **INR 1325/USD104**
37. **Trends in Anthropological Research(2022)**
    Dr. Jyoti Ratan Ghosh,Dr. Rangya Gachui **INR 399/USD 49**

Order from: Red Flower Publication Pvt. Ltd., 48/41-42, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi - 110 091(India), Mobile: 8130750089, Phone: 91-11-79695648, E-mail: info@rfppl.co.in, Website: www.rfppl.co.in

INTERNATIONAL PHYSIOLOGY

VOLUME 12 NUMBER 2-3
MAY - DECEMBER 2024

Contents

SUBSCRIPTION FORM

I want to renew/subscribe international class journal **"International Physiology"** of Red Flower Publication Pvt. Ltd.

Subscription Rates:

- Institutional: **INR 9000 / USD 664**

Name and complete address (in capitals):_____

_____

Payment detail:
Online payment link: http://rfppl.co.in/payment.php?mid=15

Cheque/DD: Please send the US dollar check from outside India and INR check from India made payable to 'Red Flower Publication Private Limited'. Drawn on Delhi branch.

Wire transfer/NEFT/RTGS:
Complete Bank Account No. 604320110000467
Beneficiary Name: Red Flower Publication Pvt. Ltd.
Bank & Branch Name: Bank of India; Mayur Vihar
MICR Code: 110013045
Branch Code: 6043
IFSC Code: BKID0006043 (used for RTGS and NEFT transactions)
Swift Code: BKIDINBBDOS

Term and condition for supply of journals
1. Advance payment required by Demand Draft payable to **Red Flower Publication Pvt. Ltd**. payable at **Delhi.**
2. Cancellation not allowed except for duplicate payment.
3. Agents allowed 12.5% discount.
4. Claim must be made within six months from issue date.

Mail all orders to
Subscription and Marketing Manager
Red Flower Publication Pvt. Ltd.
48/41-42, DSIDC, Pocket-II
Mayur Vihar Phase-I
Delhi–110 091(India).
Phone: 91-11-79695648
Cell: +91-9821671871
E-mail: sales@rfppl.co.in

BHIM BOI UPI QR
SCAN HERE TO PAY
WITH ANY BHIM UPI APP

RED FLOWER PUBLICATIONS PRIVATE LIMITED
boism-9718168299@boi

Review Article

International Physiology
Volume 12 Number 3-4, May - December 2024
DOI: https://dx.doi.org/10.21088/ip.2347.1506.122234.1

A Comprehensive Review of Autonomic Function Testing

Pooja Nigade[1], V.P. Varshney[2], Mona Bedi[3]

How to cite this article:

Pooja Nigade, V.P. Varshney, Mona Bedi. A Comprehensive Review of Autonomic Function Testing. Int. Phy.2024;12(2-3):43–49.

Abstract

The Autonomic Nervous System (ANS) regulates numerous involuntary bodily functions, including cardiovascular control, digestion, and thermoregulation. Disruptions in ANS function are associated with a diverse array of neurological and systemic disorders. Traditional autonomic testing methods, such as the Valsalva manoeuvre and orthostatic tests, primarily focus on cardiovascular reflexes, offering valuable yet indirect insights into autonomic pathways. However, technological advancements have led to the emergence of modern methods like heart rate variability (HRV) analysis, microneurography, and quantitative sudomotor axon reflex testing (QSART), which provide more precise assessments of both sympathetic and parasympathetic function. Despite their potential, the integration of these contemporary methods into routine clinical practice remains limited due to their complexity and resource requirements. This review aims to provide a comprehensive overview of classical and modern ANS testing methods, emphasising their clinical relevance in daily practice to enhance patient-care.

Aims and Methods of ANS Testing: Autonomic nervous system (ANS) testing is essential for evaluating the severity and distribution of autonomic dysfunction, diagnosing conditions such as autonomic neuropathy and orthostatic intolerance, and monitoring disease progression or treatment efficacy. Most tests assess cardiovascular reflexes in response to various stimuli, activating sympathetic or parasympathetic outflow. Classical methods, including the Ewing Battery, Head-Up Tilt (HUT) test, and Deep Breathing Test, remain clinically significant, especially in settings lacking advanced technology. Contemporary methods have revolutionised ANS testing, with HRV analysis providing in-depth insights into autonomic control over heart rate, while Microneurography and QSART assess sympathetic nerve activity and sudomotor function, respectively.

Conclusion: The evolution of autonomic function testing, from simple measures to advanced techniques, enhances our understanding of ANS regulation. Classical methods remain invaluable, yet newer technologies like HRV analysis and QSART offer detailed evaluations for complex autonomic conditions. Broad clinical integration of these contemporary methods alongside traditional approaches is essential for improving patient care.

Keywords: ANS testing, ANS, Newer methods of ANS testing.

Author's Affiliation: [1,3]Professor, [2]Postgraduate, [4]Senior Specialist, Department of General Medicine, Ballary Medical College and Research Centre, Centre, Ballari, Karnataka 583104, India.

Corresponding Author: Mona Bedi, Director Professor, Department of Physiology, Maulana Azad Medical College, MAMC & Associated Hospitals, New Delhi 110002, India.

E-mail: drmonabedi@gmail.com

Received on: 19-09-2024

Accepted on: 13-11-2024

This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0.

INTRODUCTION

The Autonomic Nervous System (ANS) controls numerous involuntary bodily functions, including cardiovascular regulation, digestion, and thermoregulation. Disruptions in ANS function are linked to a variety of neurological and systemic disorders. Historically, classical ANS testing methods, such as the Valsalva manoeuvre and orthostatic tests, have been used to assess autonomic function by measuring cardiovascular reflexes[1]. These methods, while still valuable, primarily provide indirect insights into autonomic pathways. With advancements in technology, newer methods like heart rate variability (HRV) analysis, microneurography, and quantitative sudomotor axon reflex testing (QSART) have emerged [2]. These contemporary techniques offer more detailed, objective assessments of both sympathetic and parasympathetic function and provide more accurate insights into autonomic dysfunction.

Despite the growing recognition of these advanced techniques, there remains a gap in their routine clinical integration. This is partly due to the complexity and resource demands of these tests, which are often confined to research settings or specialized centres. There is a pressing need to integrate these newer methods into everyday clinical practice, where they can complement traditional tests and offer a more comprehensive understanding of autonomic function.

This review article aims to provide a concise overview of both classical and modern methods of ANS testing, highlighting their relevance, clinical applications, and the need for broader adoption of newer techniques in routine clinical practice for enhanced patient care.

Historical Perspective

Historically, the assessment of ANS function has relied heavily on indirect measures, primarily due to the system's complexity. Early methods focused on observable physiological responses to stimuli, such as the Valsalva manoeuvre, postural changes, and the response to cold pressor test (ANS). Early pioneers such as Valsalva (1707) identified autonomic control of circulation through such manoeuvres, which remain relevant today. Similarly, the works of Jackson, King, and Ranson in the late 19th and early 20th centuries laid the foundation for recognizing how neurological diseases impair circulatory reflexes[3].

The early methods often involved simple yet clinically informative tests, such as measuring blood pressure changes in response to posture or isometric exercise (handgrip)[3]. These tests, provided valuable insights into autonomic reflexes and their dysfunction, particularly in conditions like diabetes, tabes dorsalis, and spinal cord injury.

Physiological Anatomy of ANS

The term Autonomic Nervous System (ANS) was introduced by Langley over a century ago to describe the part of the nervous system responsible for controlling visceral functions such as cardiac muscle activity[3], smooth muscle contraction, and glandular secretions. Unlike the somatic nervous system, most visceral activities regulated by the ANS occur involuntarily and cannot be consciously controlled or readily altered. The primary input to the ANS comes via somatosensory nerves, which originate from interoceptors like stretch receptors and chemoreceptors in blood vessels and visceral organs, continually monitoring the internal environment. These signals, although usually not consciously perceived, elicit responses that either stimulate or inhibit target visceral structures to maintain homeostasis.

Organization of the Autonomic Nervous System

The nervous system is divided into two parts: (1) Central nervous system (CNS) and (2) Peripheral nervous system (Fig. 1). The peripheral nervous system (PNS) is further divided into somatic, autonomic and enteric nervous system. The CNS receives all the peripheral sensory information via the sensory nerve fibres, also called the afferents and sends the motor information to the effectors via motor nerve fibres, also called efferent fibres. These efferent fibres travel through the somatic and the ANS. The input for ANS is via somatic afferent pathway which provides information to the CNS that in turn stimulates the ANS. This would lead to an involuntary action. The ANS is responsible for involuntary motor responses. The effector may be smooth or cardiac muscle (both involuntary muscles) or a gland. Thus, the **ANS is a visceral efferent system**, which means it sends motor impulses from the CNS to the visceral organs. It functions automatically and continuously, without conscious effort, to innervate smooth muscle, cardiac muscle, and glands. The ANS has two parts, sympathetic division and a parasympathetic division. The sympathetic and the parasympathetic fibers are the efferents that connect CNS to the visceral organs. There

is a fine balance between these two systems is required to maintain homeostasis. ENS comprises sympathetic and parasympa-thetic fibers that regulate the activity of the gastrointestinal tract. The sympathetic and the parasympathetic efferent pathways are composed of two neurons: (1) **Preganglionic Neurons:** Originating in the CNS, their axons extend to an autonomic ganglion and (2) **Postganglionic Neurons:** These neurons relay signals from the ganglion to the target organ.

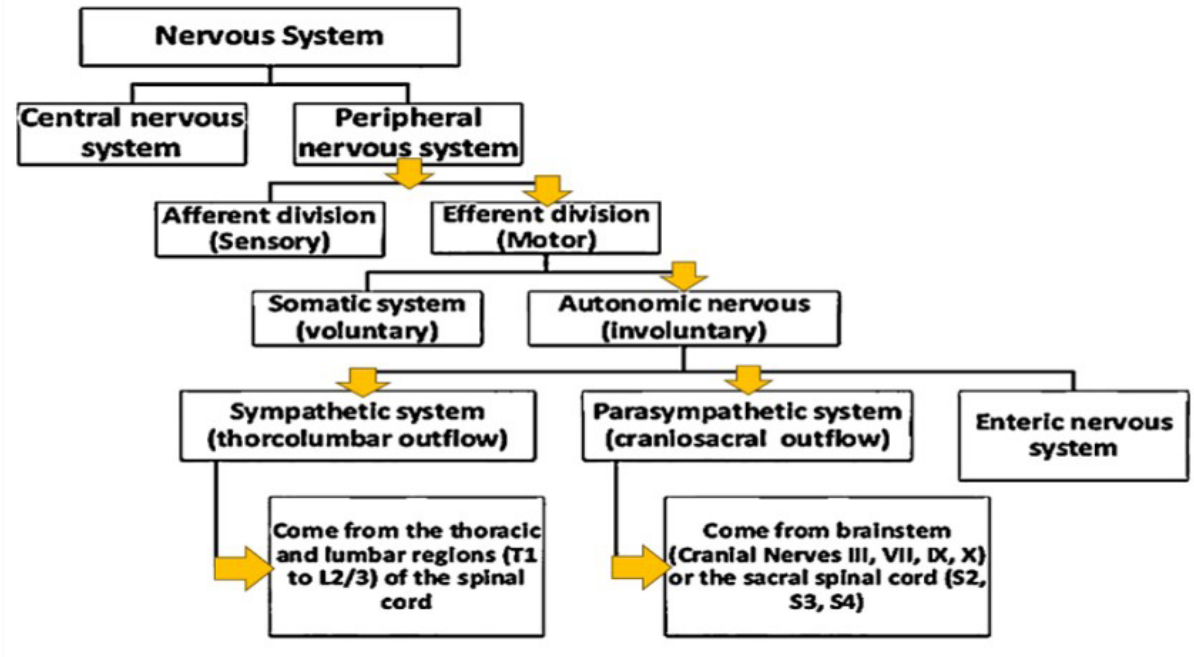


Fig. 1: Organization of ANS

Functions of the ANS

(a) Parasympathetic System (PSS): The parasympathetic division is responsible for conserving and storing energy, promoting anabolic processes during restful states.

(b) Sympathetic System (SS): The sympathetic division, on the other hand, is catabolic and activates the body for emergency responses. Its effects are generally prolonged and widespread, whereas the parasympathetic responses are more localized and transient.

Aims of ANS Testing:

- To evaluate the severity and distribution of autonomic function
- To diagnose limited autonomic neuropathy
- To diagnose and assess orthostatic intolerance
- To monitor the course of dysautonomia
- To monitor response to treatment
- To serve as an instrument in research studies

Most of the tests are based on evaluation of the cardiovascular reflexes triggered by performing specific provocative manoeuvres. Stimuli that raise blood pressure, such as isometric exercise, cold pressor test or mental arithmetic, activate mainly sympathetic outflow. Moreover, blood pressure responses to orthostatic testing and Valsalva manoeuvre are in a large part a reflection of sympathetic activity.[4-6] HR Changes during orthostatic testing and Valsalva manoeuvre, as well as during deep breathing or diving reflex, reflect parasympathetic modulation.[6,7]

Traditionally, batteries of autonomic tests have been introduced, with the Ewing battery being the most popular.[8] **Ewing Battery:** The Ewing battery, developed by Ewing and Clarke, is a comprehensive set of tests used to assess both parasympathetic and sympathetic function. It focuses on cardiovascular reflex responses, including heart rate and blood pressure variations. The tests involve measuring responses to autonomic stimuli, such as deep breathing, the Valsalva manoeuvre, and postural changes. They are widely used to detect autonomic failure in diabetic patients and can reveal abnormalities related to both parasympathetic and sympathetic nervous systems.[8]

More recently, new techniques, such as evaluation of heart rate variability or microneurography, have been introduced as diagnostic tools.

Assessment of Autonomic Nervous System (ANS) Function

The assessment of ANS function has evolved significantly, progressing from classical methods to contemporary advanced techniques. Autonomic function tests (AFT) are essential in confirming and determining the severity of dysautonomia, as well as identifying structural and functional deficits in the ANS. These tests are crucial in both clinical and research settings, with various techniques developed to evaluate different autonomic pathways.

Classical Methods for ANS Testing

Older methods of ANS testing remain clinically significant, particularly in the diagnosis of autonomic dysfunctions such as diabetic neuropathy, orthostatic hypotension, and cardiovascular dysregulation. These techniques, though simpler than modern methods, continue to provide essential diagnostic insights, especially in settings where advanced technology may not be readily available.

The followings are the classical/older methods of ANS Testing:

1. **Head Up Tilt (HUT) / Lying-Standing Test (LST):** The head-up tilt and lying-standing tests evaluate the cardiovascular response to positional changes, making them critical in diagnosing orthostatic hypotension. By transitioning from lying to standing, blood pools in the lower extremities, challenging the body's ability to maintain blood pressure through autonomic reflexes. A significant drop in systolic or diastolic pressure can signal autonomic failure. HUT is often combined with other autonomic tests for a more robust evaluation of disorders like postural tachycardia syndrome (POTS)[8].
2. **Deep Breathing Test:** This test measures heart rate variability in response to deep breathing, exploiting the natural respiratory sinus arrhythmia. Normally, the heart rate increases during inspiration and decreases during expiration, reflecting parasympathetic activity. A reduction in heart rate variability during this test is often an indicator of impaired parasympathetic function, as seen in conditions like diabetic autonomic neuropathy[9].
3. **Valsalva Manoeuvre** The Valsalva manoeuvre is a cornerstone of autonomic testing, used to assess baroreceptor reflexes and both parasympathetic and sympathetic control. It involves forced expiration against a closed airway, creating distinct cardiovascular phases that can reveal autonomic dysfunction. Patients with ANS disorders often show abnormal blood pressure recovery and heart rate responses during this test[9].
4. **Isometric Handgrip Test** During the isometric handgrip test, patients sustain an isometric contraction by squeezing a dynamometer, leading to an increase in diastolic blood pressure due to sympathetic outflow. A blunted rise in blood pressure may indicate defective sympathetic control, which is commonly associated with cardiovascular autonomic neuropathy[10].
5. **Cold Pressor Test** In this test, a patient immerses their hand or foot in cold water, which induces a sympathetic response, resulting in elevated heart rate and blood pressure. This test evaluates sympathetic vasomotor function and is particularly useful in assessing autonomic responses to thermal and pain stimuli[10].

The classical ANS tests can be clubbed in to those for sympathetic and parasympathetic testing for the ease to understand as given in Table 1.

Table 1: Different types of classical ANS tests (Sympathetic and Parasympathetic)

Method	Type	Description	Clinical Relevance
Cold Pressor Test	Sympathetic	Patient immerses hand or foot in cold water, including a sympathetic response.	Assesses sympathetic vasomotor function; useful for evaluating autonomic responses to thermal and pain stimuli.
Isometric Handgrip Text	Sympathetic	Patient squeezes a dynamometer to induce an increase in diastolic blood pressure due to sympathetic outflow.	Evaluates sympathetic control of blood pressure; commonly used to assess cardiovascular autonomic neuropathy.
Head-Up Tilt Text (HUT)	Sympathetic	Similar to HUT, accesses blood pressure changes when moving from lying to standing.	Used for diagnosing orthostatic hypotension and autonomic dysfunction.

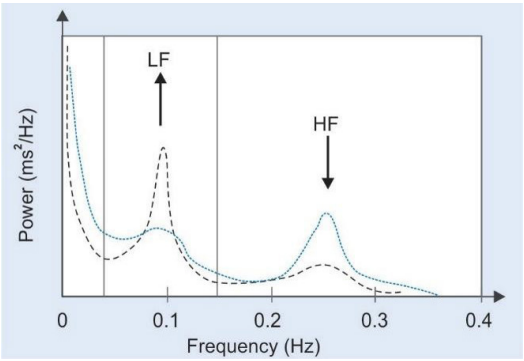
Method	Type	Description	Clinical Relevance
Deep Breathing Text	Parasympathetic	Meaures heart rate variability in response to deep breathing, reflecting respiratory sinus arrhythmia.	Accesses parasympathetic function; reduced variability indicates impaired parasympathetic control.
Valsalva Manoeuvre	Sympathetic & Parasympathetic	Patient Performs forced expiration against a closed airway, accessing baroreceptor reflexes and autonomic control.	Provides insights inot both sympathetic and parasympathetic functions, revealing abnormalities in blood pressure recovery and heart rate responses.

A. Contemporary Methods for ANS Testing

1. With advancements in technology, newer methods have been developed to provide more detailed and precise evaluations of autonomic function. These methods offer greater insight into specific components of the ANS and are increasingly being used for both diagnostic and research purposes.
2. Heart Rate Variability (HRV) Analysis The variations in HR can be evaluated by the following two HRV indices[15]:
   A. **Time Domain Analysis:** A continuous ECG record is taken and a normal to normal (N-N interval i.e. all intervals between adjacent QRS complexes,the standard deviation of RR intervals (SDNN) and the root mean square of successive differences (rMSSD) are determined.
   B. **Frequency Domain Analysis**

   It analyses different frequency components of the waveform. The main frequency components that represent autonomic activity are:

- High frequency (HF)—0.15–0.4 Hz
- Low frequency (LF)—0.04–0.15 Hz
- Very low frequency (VLF)—0.0–0.04 Hz



Frequency domain indices of HRV analysis. (HF: high frequency; HRV: heart rate variability; LF: low frequency).[15]

- The LF and HF components are relative indices of cardiac sympathetic and vagal activity, respectively.
- HF component is because of vagal tone during respiratory cycle.
- Low frequency component results from self-oscillation in the sympathetic component of the baroreceptor reflex loop as a result of negative feedback.

3. **Microneurography:** Introduced in the 1960s, microneurography involves inserting fine electrodes into peripheral nerves to record sympathetic nerve activity. This highly specialized technique provides direct insights into sympathetic outflow to various organs, such as the skin and muscles. It is particularly useful for research and clinical evaluation of conditions such as hypertension and diabetic neuropathy. In patients with hypertension, microneurography has demonstrated its ability to accurately assess sympathetic nerve activity, which can guide therapeutic strategies.[12]
4. **Quantitative Sudomotor Axon Reflex Test (QSART):** QSART assesses sudomotor function by measuring sweat production in response to acetylcholine iontophoresis. This test is particularly useful in diagnosing small-fibre neuropathies and other conditions that affect sweat gland function. It is particularly beneficial for patients with suspected peripheral autonomic dysfunction, such as those with diabetic neuropathy or small-fibre neuropathy. QSART provides precise insights into sweat gland function, which is crucial for diagnosing these conditions.[12]
5. Baroreflex Sensitivity Testing Baroreflex sensitivity (BRS) is a key measure of autonomic control over blood pressure. Traditional methods use pharmacological agents like phenylephrine to manipulate blood pressure, followed by analysis of heart rate responses. Newer, non-invasive methods use spontaneous fluctuations in blood pressure and HR to assess BRS. This technique has clinical utility in diagnosing syncope and

other autonomic disorders. Many clinical uses of this test have emerged, e.g. vasovagal syncope, impaired baroreflex sensitivity.[13]

6. **Head-Up Tilt Test (HUTT)** HUTT is predominantly used in the evaluation of syncope. Unlike active standing, which can mask autonomic dysfunction through compensatory mechanisms, HUTT provides a controlled environment for assessing cardiovascular responses to orthostasis. It is particularly useful in diagnosing vasovagal syncope and orthostatic hypotension.[13]
7. **Sympathetic Skin Response (SSR)** SSR is a simple, non-invasive test that measures the electrical potentials generated by sweat gland activity in response to stimuli such as noise or electrical stimulation. It provides an assessment of cholinergic sympathetic function and is often used in the diagnosis of disorders such as diabetic neuropathy and Guillain-Barré syndrome.[13]

Newer AFT methods offer significantly improved diagnostic accuracy compared to older techniques. HRV analysis has demonstrated a sensitivity of 90% and specificity of 85% in detecting autonomic dysfunction, particularly in cardiovascular conditions like heart failure, whereas traditional tests like the Valsalva manoeuvre show lower sensitivity, around 70-75%. Similarly, microneurography, which directly measures sympathetic nerve activity, achieves 95% diagnostic accuracy in conditions like hypertension, outperforming older orthostatic tests with a sensitivity of only 65-70%. Additionally, the Quantitative Sudomotor Axon Reflex Test (QSART) exhibits a sensitivity of 92% and specificity of 89% for diagnosing small fibre neuropathy, surpassing the cold pressor test, which has a sensitivity of 60-70% for similar dysfunctions.[14]

FUTURE DIRECTIONS

The field of ANS testing continues to evolve, with new technologies being developed to improve accuracy and reduce invasiveness, which gives an extremely high Value: Risk ratio. Non-linear analysis of HRV, for example, is gaining traction as a more sophisticated method for assessing autonomic modulation. Similarly, advances in wearable technology may soon allow continuous monitoring of autonomic function in real-world settings, providing valuable insights into disease progression and treatment efficacy.

CONCLUSION

AFT is an essential component of diagnosing and managing a variety of conditions, particularly those affecting the cardiovascular system. Both classical and modern methods have their place in clinical practice. AFT can non-invasively evaluate the severity and distribution of autonomic failure. They have sufficient sensitivity to detect even subclinical dysautonomia. As technology advances, the integration of these methods into routine care promises to enhance our understanding of autonomic dysfunction and improve patient outcomes.

REFERENCES

1. Spalding JMK. Disorders of the autonomic nervous system. J Neurol Neurosurg Psychiatry. 1966; 35:276-91.
2. Freeman R, Wieling W, Axelrod FB, Benditt DG, Benarroch E, Biaggioni I, *et al.* Autonomic nervous system testing: a review of the literature and current clinical applications. Neurology. 2013; 80(1):1-13.
3. Benarroch EE. The autonomic nervous system: basic anatomy and physiology. Continuum (Minneap Minn). 2013; 19(1):19-39.
4. Zygmunt A, Stanczyk J. Methods of evaluation of autonomic nervous system function. Arch Med Sci. 2010 Feb; 6(1):11-8. DOI: 10.5114/aoms.2010.13500.
5. Shaffer F, Ginsberg JP. An overview of heart rate variability metrics and norms. Front Public Health. 2017;5:258.
6. Lombardi F. Clinical utility of heart rate variability: perspectives from the European Society of Cardiology Task Force. Card Electrophysiol Rev. 2002; 6(3):245-9.
7. Malik M. Heart rate variability: standards of measurement, physiological interpretation, and clinical use. Circulation. 1996;93(5):1043-65.
8. Ewing DJ, Clarke BF. "Diagnosis and management of diabetic autonomic neuropathy." Br Med J. 1982; 285(6346):916-918.
9. Low PA, Tomalia VA. "Clinical autonomic testing: then and now." Clin Auton Res. 2015; 25(2):85-89.
10. Freeman R. "Autonomic peripheral neuropathy." Lancet. 2005; 365(9466):1259-1270.
11. Malliani A, Pagani M, Lombardi F. "Cardiovascular neural regulation explored in the frequency domain." Circulation. 1991; 84(2):482-492.
12. Ewing DJ, Hume L, Campbell IW, Murray A, Neilson JM, Clarke BF. "Autonomic mechanisms in the initial heart rate response to standing." J Appl Physiol Respir Environ Exerc Physiol. 1980; 49(5):809-814.

13. Valsalva AM. De aure humana tractatus. Utrecht: Traj ad Rhenum; 1707.
14. Johnson RH, Lee GDJ, Oppenheimer DR, Spalding JMK. Autonomic function in cerebrovascular accidents. Lancet. 1966; 1(731):276-80.
15. Varshney VP, Bedi M.Ghai's textbook of practical Physiology, 10th Edition, 2023;2.29;186-91

Review Article

International Physiology
Volume 12 Number 3-4, May - December 2024
DOI: https://dx.doi.org/10.21088/ip.2347.1506.122234.2

Battling the Balance: Oxidative Stress and the Power of Antioxidants

Amitava Khan

How to cite this article:

Amitava Khan, Battling the Balance: Oxidative Stress and the Power of Antioxidants. Int.Phy.2024;12(2-3):50–54.

Abstract

Background: Oxidative stress results from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidative defenses. ROS, derived from oxygen, are essential in cellular signaling but can also damage cellular macromolecules, including lipids, proteins, and DNA, when produced excessively. This imbalance is associated with aging and a range of diseases, including atherosclerosis, neurodegenerative disorders, diabetes, and cancer.

Aims: The aim of this article is to investigate the mechanisms of oxidative stress, its biological effects, and the role of antioxidants in mitigating oxidative damage. Additionally, the article explores the implications of oxidative stress in various diseases and potential therapeutic strategies.

Materials and Methods: The article involves a comprehensive review of existing literature to elucidate the sources and types of ROS, their mechanisms of action, and the body's enzymatic (e.g., superoxide dismutase, catalase) and non-enzymatic (e.g., vitamins C and E, glutathione) antioxidant defenses. Special attention is given to the biochemical pathways of ROS formation and detoxification.

Results: Findings indicate that ROS are naturally produced during cellular metabolism and play dual roles in physiological and pathological processes. Under stress conditions or exposure to environmental toxins, ROS production overwhelms antioxidant defenses, leading to lipid peroxidation, protein oxidation, and DNA damage. Antioxidants mitigate this damage by neutralizing ROS, maintaining cellular integrity, and reducing the risk of oxidative stress-related diseases.

Conclusion: Oxidative stress plays a pivotal role in aging and the development of chronic diseases. Antioxidants, both endogenous and exogenous, are crucial in managing oxidative damage. Enhancing antioxidant defenses through dietary intake or pharmacological interventions may provide therapeutic benefits and prevent oxidative stress-related pathologies. Further research into targeted antioxidant therapies is recommended to improve health outcomes.

Keywords: Oxidative Stress, Reactive Oxygen Species, Antioxidants, Chronic Diseases.

Author's Affiliation: Department of Physiology, Sonarpur Mahavidyalaya, Rajpur, Kolkata, West Bengal 700149, India.

Corresponding Author: Amitava Khan, Department of Physiology, Sonarpur Mahavidyalaya, Rajpur, Kolkata, West Bengal 700149, India.

E-mail: amitava84@gmail.com

Received on: 28-11-2024

Accepted on: 28-12-2024

This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0.

INTRODUCTION

Oxidative stress is a multifaceted biological phenomenon characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them through antioxidative systems. ROS, a collective term for chemically reactive molecules containing oxygen, play a dual role in human physiology. They are essential for normal cellular signaling and host defense mechanisms. On the other hand, their overproduction or insufficient detoxification can lead to cellular and molecular damage. This damage arises from the toxic effects of peroxides and free radicals, which attack essential cellular components such as lipids, proteins, and nucleic acids, thereby impairing cell function and viability.

Antioxidative systems encompass a range of enzymatic and non-enzymatic mechanisms that work to detoxify reactive intermediates or repair the damage caused by oxidative stress. These systems are critical in maintaining cellular homeostasis and preventing the harmful effects of ROS. Enzymatic antioxidants, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, play significant roles in converting harmful ROS into less reactive molecules. Non-enzymatic antioxidants, including vitamins C and E, glutathione, and carotenoids, further contribute to reducing oxidative damage by scavenging free radicals and terminating their chain reactions.

In humans, oxidative stress has been implicated in the pathogenesis of numerous diseases. For example, it contributes to the development of cardiovascular diseases such as atherosclerosis by promoting lipid peroxidation and endothelial dysfunction. Similarly, in neurodegenerative conditions like Alzheimer's and Parkinson's diseases, oxidative stress accelerates neuronal damage and death. Other chronic conditions, including diabetes mellitus, cancer, inflammatory disorders, and certain psychological diseases, have also been strongly linked to oxidative stress. Moreover, it is considered a key factor in the aging process, as the cumulative damage from ROS over time contributes to the decline in cellular and organ function associated with aging.[1]

Understanding the mechanisms of oxidative stress and its impact on health has become a critical area of research. Insights into how ROS production is regulated and how antioxidative defenses can be enhanced offer potential strategies for mitigating the detrimental effects of oxidative stress. These approaches may pave the way for novel therapeutic interventions to combat oxidative stress-associated diseases and promote healthy aging.

Free radicals and reactive oxygen species

Free radicals in an atom or group of atoms have one or more unpaired electrons. Free radicals can have positive, negative or neutral charge. They are formed as necessary intermediates in a variety of normal biochemical reactions but when generated in excess or not appropriately controlled, an extensive range of macromolecules. A well-known feature of free radicals is that they have extremely high chemical reactivity which is responsible for their biological activity as well as damaging effect on cells[2].

Oxygen radicals

There are many types of free radicals but those most concerned in the biological system are derived from O_2 and collectively known as ROS. Sequential reduction of molecular O_2 leads to the formation of a group of ROS:

- Super oxide anion (O_2^-)
- Peroxide (O_2^{2-})
- Hydroxyl radicals ($^{\bullet}OH$)

Another radicals derived from O_2 is singlet oxygen ($^{\bullet}O_2$).This is an excited form of O_2 in which one of the electrons jump to superior orbital following absorption of energy[2].

Formation of ROS by stress

O_2 derived radicals are derived constantly as part of normal aerobic life. They are formed in mitochondria as O_2 is reduced along the electron transport chain. ROS are also formed as necessary intermediates in a variety of reactions e.g. WBC like neutrophils constantly produce free radicals which are used in host defence to kill invading pathogen. Cell exposed to abnormal environments such as hypoxia generate abundant and often damaging ROS. A number of drugs have oxidising effects on cells and lead to production of O_2 radicals. Stress is well known to generate O_2 within biological system[1]. It generates ROS such as superoxide anions, hydrogen peroxide, and hydroxyl radicals, which show high reactivity to a variety of cellular macromolecules, including DNA, lipids and proteins. The damage caused by oxidative stress is either direct interaction with target molecules or indirectly by the formation of ROS, resulting from the radiolysis of water. As human tissues contain 80% water, the major radiation damage is due generation of ROS.

Biological effects of ROS

ROS are generated in a number of reactions, are essential to life. Evidence suggests that ROS are involved in cellular signaling and can function as mitogens. Yet, despite their beneficial effect, ROS can be toxic for cells. They damage all macromolecules including lipids, proteins and nucleic acids due to the presence of unpaired electrons (e^-).

Free radicals damage in 3 ways[2] :

- **By lipid peroxidation**: The free radicals attack the double bond of unsaturated fatty acids of membrane phospholipids and damage the cellular membranes.
- **By oxidative modification of protein**: The amino acids of protein get oxidized in contact with free radicals causing physical changes in the protein molecules including fragmentation and aggregation. This increases the susceptibility of the protein molecules to proteolytic degradation and thereby damaging functioning of the cell and its organelles.
- **By modification of genomic or cellular structure**: Free radicals cause the lesion of thymine and/or cytosine bases thereby breaking single or double stranded DNA and modify genomic DNA consequently cellular structure. (Figure 1)

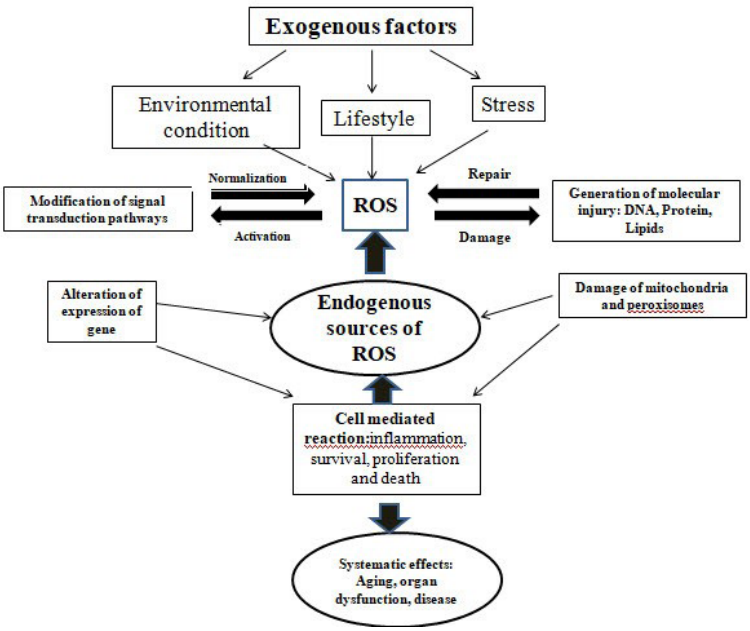


Fig. 1: Mechanism and effects of Reactive Oxygen Species (ROS) in cell

Mechanism of protection against free radicals

Life on earth evolved in the presence of free O_2 and necessarily adapted by evolution of large battery of the antioxidant system. Some of these antioxidants are present in all types of life forms; from bacteria to mammals, indicating their appearance early in the history of life.

Antioxidants

An antioxidant is a molecule capable of slowing or preventing oxidation of other molecules. Oxidation reactions can produce free radicals, which starts chain reactions that damage the cells. Antioxidants terminate these chain reactions by oxidising themselves.

Although oxidation reactions are crucial for life, they can also be damaging. Hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, vitamin E as well as enzymes such as catalase, SOD and various peroxidases. Low level of antioxidants enzymes cause the oxidative stress and may damage to kill the cells.

a) Enzymatic antioxidants: These groups of enzymes play significant roles in protecting from oxidative stress.

Super oxide dismutase (SOD): These are enzymes that catalyze the conversion of two superoxides to H_2O_2 and O_2. The benefit here is that, H_2O_2 is substantially less toxic than super oxide (O_2^-). SOD accelerates this detoxifying reaction roughly 10,000 fold over non catalyzing reaction.

SOD are the metal containing enzyme that depends on the Mn, Cu, or Zn for their antioxidant

activity. In mammals, Mn containing enzyme is most abundant in the mitochondria, while Zn and Cu containing enzyme forms predominate in the cytoplasm. Interestingly SOD is an inducible enzyme. Exposure of bacteria or vertebrate cells to higher concentration of O_2 results in rapid increase in concentration of SOD[3].

Catalase: This is formed in peroxisomes of eukaryotic cells. It degrades H_2O_2 and O_2 and hence finishes the detoxification reaction started by SOD[3].

Glutathione peroxidase: This is a group of enzymes the most abundant of which contain Se. These enzymes like catalase degrade H_2O_2. They also reduce organic peroxidase to alcohol providing another route for eliminating toxic O_2[4].

Nitric oxide synthase (NOS): NOS is an enzyme in the body that contributes to transmission from one neuron to another, to the immune system and dilating blood vessels. It does so by the synthesis of nitric oxide (NO) from terminal N_2 atom of L-Arginine in the presence of NADPH and dioxygen. NOS is the only known enzyme that binds FAD, FMN, heme, and calmodulin. NOS activates cyclic GMP, which induce smooth muscle relaxation by multiple mechanisms[5]. There are 3 types of NOS; neuronal NOS (nNOS),which was originally identified in nervous system tissues and is present all the time in the cells, endothelial NOS (eNOS) which is also expressed constitutively in the endothelial cells and synthesizes NO needed for the regulation of blood pressure, and inducible NOS (iNOS) which is found at the site of chronic inflammation.

b) Non enzymatic antioxidant:

The non enzymatic antioxidants having particular importance are:

Vitamin E: This is the major lipid soluble antioxidant and plays a vital role in protecting membranes from oxidative damage. Its primary activity is to trap peroxyradicals in cellular membranes.

Vitamin C: Also called Ascorbic acid, is a water soluble antioxidant that can reduce free radicals from a variety of sources. It also appears to participate in recycling vitamin E radicals.

Glutathione: It may be the most important intracellular defence against damage by ROS. It is tri-peptide (glutamyl-cysteinyl-glycine). These cystein provides protection against exposed free radicals i.e. very reactive, providing an abundant target for free radical attacks. Reaction with radicals oxidise glutathione, but the reduced form is regenerated in a redox cycle involving glutathione reductase and electron acceptor NADPH[6].

Carotenoids (β-Carotene)

These are mainly colored pigments present in plants and microorganisms. Epidemiological studies have revealed that a diet rich in carotenoids is correlated with a lower risk of age-related diseases[7]. Primarily, β-carotene has been found to react with peroxyl (ROO.) to prevent damage in lipophilic compartments hydroxyl (.OH), and superoxide radicals[8]. The antioxidant activity of carotenoids arises due to their ability to delocalize unpaired electrons, and thus quench singlet oxygen without degradation. The efficacy of carotenoids with respect to physical quenching is related to the number of conjugated double bonds present in the molecule. Both β-Carotene and retinoic acid are capable of regulating different transcription factors, β-Carotene inhibits the oxidant-induced NF-κB activation and interleukin (IL)-6 and tumor necrosis factor-α production. On the other hand, retinoic acid can affect cell, arrest cell cycle, or both[9-13].

Melatonin

This is a neurohormone that is derived from tryptophan mainly in the pineal gland. One of the major functions of melatonin is scavenging free radicals in oxygen metabolism, thereby potentially protecting against free radical-induced damage to DNA, proteins and membranes. Owing to these properties, it has the potential to play an important role in the reduction of free radical-mediated diseases[10].

In addition to these big three, there are numerous other small molecules that act as antioxidant eg. bilirubin, uric acid etc.

CONCLUSIONS

Present research has led to a universal agreement that oxidative damage to proteins, lipids, and DNA occurs as a result of ROS overproduction. These are highly reactive due to the unpaired electrons in their structure that allow them to react with several biological macromolecules in cell, thus altering their functions. ROS are produced by cellular metabolic reactions that use oxygen and shift the balance in oxidant/antioxidant status in favour of the oxidants. A variety of environmental factors, such as air pollutants or cigarette smoke, can result in the production of ROS, which can also affect

the expression of several genes by upregulation of redox-sensitive transcription factors and chromatin remodelling through alteration in histone acetylation/deacetylation. The human body deals with the pathological effects of ROS by utilizing the endogenous antioxidant enzymatic system and by the ingestion of exogenous antioxidants in the diet. If the oxidative stress exceeds the protection afforded by antioxidants, the aging process and some of the diseases associated with it such as cardiovascular diseases, neurodegenerative diseases, diabetes and cancer can accelerate. Regulation of redox state is critical for cell viability, activation, and proliferation, as well as organ function.

Conflict of Interest: None

Funding: None

REFERENCES

1. Rhodes CJ. In Toxicology of the Human Environment-the critical role of free radicals (Taylor & Francis, London) 2001.
2. Metzler DE. In Biochemistry- the chemical reaction of living cell (Academic Press, California, US) 2001: 10.
3. Meister A. Glutathione-Ascorbic acid antioxidant system in animal. J Biol.Chem. 1994; 269(13): 9397-9400.
4. Stamler JS. Redox signalling:nitorsylation and related target interactions of NO. Cell. 1994;78(6): 931-936.
5. Mettler FAJ, Voelz GL. Major radiation exposure-what to expect and how to respond. N Eng J Med. 2002; 346(20): 1554-1561.
6. Khalid R. Studies on free radicals, antioxidants, and co-factors. Clinical Interventions in Aging. 2007; 2(2): 219-236.
7. El-Agamey A, Lowe GM, McGarvey DJ, Mortensen A, Phillip DM, Truscott TG. Carotenoid radical chemistry and antioxidant/pro-oxidant properties. Arch. Biochem. Biophys. 2004; 430(1):37-48.
8. Niles RM. Signaling pathways in retinoid chemoprevention and treatment of cancer. Mutat Res. 2004; 555(1-2): 81-96.
9. Donato LJ, Noy N. Suppression of mammary carcinoma growth by retinoic acid: proapoptotic genes are targets for retinoic acid receptor and cellular retinoic acid-binding protein II signalling. Cancer Res. 2005; 65(18): 8193-8899.
10. Niizuma H, Nakamura Y, Ozaki T, Nakanishi H, Ohira M. Bcl-2 is a key regulator for the retinoic acid-induced apoptotic cell death in neuroblastoma. Oncogene. 2006; 25(36):5046-5055.
11. Khan A, Manna K, Bose C, Sinha M, Das DK, Kesh SB, Chakrabarty A, Banerji A, Dey S. Gossypetin, a naturally occurring hexahydroxy flavone, ameliorates gamma radiation-mediated DNA damage. International Journal of Radiation Biology. 2013; 89(11): 965-975.
12. Khan A, Manna K, Das DK, Kesh SB, Sinha M, Das U, Biswas S, Sengupta S, Sikder K, Chakrabarty A, Banerji A, Dey S. Gossypetin ameliorates ionizing radiation-induced oxidative stress in mice liver—a molecular approach. Free Radical Research. 2015; 49(10): 1173-1186.
13. Khan A, Manna K, Bose C, Das DK, Sinha M, Kesh SB, Das U, Dey RS, Dey S. Seabuckthron (*Hippophae rhamnoides* L.) leaf extract ameliorates the gamma radiation mediated DNA damage and hepatic alterations. Indian journal of experimental biology. 2014; 52:952-964.

Instructions to Authors

Submission to the journal must comply with the Guidelines for Authors.
Non-compliant submission will be returned to the author for correction.

To access the online submission system and for the most up-to-date version of the Guide for Authors please visit: http://www.rfppl.co.in

Technical problems or general questions on publishing with **IP** are supported by Red Flower Publication Pvt. Ltd.'s Author Support team (http://rfppl.co.in/article_submission_system.php?mid=5#)

Alternatively, please contact the Journal's Editorial Office for further assistance.

Editorial Manager
Red Flower Publication Pvt. Ltd.
48/41-42, DSIDC, Pocket-II
Mayur Vihar Phase-I
Delhi - 110 091(India)
Mobile: 9821671871, Phone: 91-11-79695648
E-mail: author@rfppl.co.in

Review Article

International Physiology
Volume 12 Number 3-4, May - December 2024
DOI: https://dx.doi.org/10.21088/ip.2347.1506.122234.3

Novel Approach of Ameliorative Role of Different Vitamins in the Prevention of Cardiovascular Diseases

Ankush Sharma[1], Shubhadeep Chakraborty[2], Surajit Bhattacharjee[3]

How to cite this article:

Ankush Sharma, Shubhadeep Chakraborty, Surajit Bhattacharjee, Novel Approach of Ameliorative Role of Different Vitamins in the Prevention of Cardiovascular Diseases. Int.Phy.2024;12(2-3):56-63.

Abstract

Cardiovascular diseases (CVDs) represent a significant global health burden, necessitating innovative approaches for prevention and treatment. This review explores the multifaceted roles of vitamins specifically Vitamins C, D, E, K, and B complex—in maintaining cardiovascular health. Vitamins contribute to critical physiological processes, including antioxidant defense, inflammation modulation, vascular integrity, and lipid metabolism. Vitamin C enhances endothelial function and reduces oxidative stress, while Vitamin D regulates calcium homeostasis and mitigates arterial stiffness. Vitamin E prevents LDL oxidation and supports endothelial health. The B-complex vitamins lower homocysteine levels, reducing thrombotic risk, and Vitamin K prevents vascular calcification. Despite promising evidence, conflicting results from clinical trials underscore the need for personalized supplementation strategies and further research to establish definitive guidelines. This review highlights the potential of vitamins as complementary agents in CVD care, emphasizing the importance of balanced intake for cardiovascular health.

Keywords: Cardiovascular Diseases, Vitamins, Antioxidant Defense, Endothelial Function, Lipid Metabolism.

INTRODUCTION

Cardiovascular diseases (CVDs) are a leading cause of morbidity and mortality worldwide, contributing to nearly 32% of global deaths annually (Di Cesare *et al*., 2024). These diseases encompass a wide spectrum, including coronary artery disease, hypertension, stroke, and heart failure, which are influenced by genetic, environmental, and lifestyle factors. Despite advancements in medical treatments, the burden of CVDs remains significant, prompting the exploration of complementary and preventive strategies to mitigate risks and improve outcomes. Among these, the role of vitamins has gained considerable attention due to their potential in modulating physiological processes relevant to cardiovascular health.

Vitamins, as essential micronutrients, participate in critical cellular and biochemical functions. They are involved in antioxidant defense, modulation

Author's Affiliation: [1]Department of Education, Henry Derozio Academy, Kunjaban, Tripura 799006, India, [2]Department of Physics, Bir Bikram Memorial College, College Tilla, Tripura 799004, India, [3]Department of Biological Sciences, Bodhjung Higher Secondary School, Agartala, Tripura 799001, India.

Corresponding Author: Surajit Bhattacharjee, Department of Biological Sciences, Bodhjung Higher Secondary School, Agartala, Tripura 799001, India.

E-mail: surajit.hptu@gmail.com

Received on: 21-01-2025 **Accepted on:** 07-03-2025

This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0.

of inflammation, vascular health, and lipid metabolism, all of which are pivotal in maintaining cardiovascular integrity (Marchioli *et al.,* 2001). For instance, oxidative stress, a key contributor to atherosclerosis and other CVDs, is characterized by an imbalance between free radicals and antioxidants. Vitamins such as Vitamin C and E, which possess potent antioxidant properties, may counteract oxidative damage by scavenging reactive oxygen species (Münzel *et al.,* 2017).

Vitamin C has been widely studied for its role in enhancing endothelial function, reducing arterial stiffness, and mitigating hypertension. Research indicates that regular intake of Vitamin C can improve nitric oxide bioavailability, which helps in vasodilation and reduces the risk of endothelial dysfunction, a precursor to atherosclerosis (May & Harrison, 2013). Similarly, Vitamin E, another lipid-soluble antioxidant, has shown promise in preventing the oxidation of low-density lipoprotein (LDL) cholesterol, a critical step in plaque formation within arterial walls (Catalgol & Ozer, 2011). In addition to antioxidant effects, other vitamins such as Vitamin D have emerged as key regulators of cardiovascular health. Vitamin D is essential for calcium homeostasis and bone health but also exerts significant effects on the cardiovascular system. Deficiency of Vitamin D has been associated with an increased risk of hypertension, myocardial infarction, and heart failure, possibly through mechanisms involving the renin-angiotensin-aldosterone system and inflammatory pathways (Nardin *et al.,* 2024). Clinical studies suggest that adequate levels of Vitamin D may reduce arterial stiffness and improve overall cardiac function (de la Guía-Galipienso *et al.,* 2021). The B complex vitamins, including B_6, B_9 (folate), and B_{12}, play a crucial role in homocysteine metabolism. Elevated levels of homocysteine are a known risk factor for CVDs, as they can damage endothelial cells and promote thrombogenesis. Supplementation with these vitamins has been shown to lower homocysteine levels and potentially reduce the risk of cardiovascular events (Mohan *et al.,* 2023). Furthermore, Vitamin K, known for its role in coagulation, also contributes to vascular health by regulating calcium deposition in arteries. Adequate Vitamin K levels help prevent vascular calcification, a common feature of advanced atherosclerosis and arterial stiffness (van Gorp *et al.,* 2021). Despite the potential benefits, the use of vitamins in CVD prevention and treatment remains a subject of ongoing research and debate. While observational studies and some clinical trials highlight positive associations, other large-scale randomized controlled trials (RCTs) have yielded inconclusive or conflicting results. Factors such as dosage, bioavailability, patient population, and study design contribute to the variability in outcomes (Simsek *et al.,* 2021). Moreover, excessive intake of certain vitamins, particularly fat-soluble ones like Vitamin E and K, may pose risks such as hypervitaminosis or adverse interactions with medications (Reddy and Jialal 2022).

This systematic review aims to provide a comprehensive analysis of the role of vitamins—specifically Vitamin C, D, E, B complex, and K—in the prevention and management of CVDs. By synthesizing current evidence on their mechanisms of action, clinical efficacy, and associated risks, this review seeks to elucidate the potential of vitamins as adjunctive agents in cardiovascular care. Furthermore, it highlights the gaps in existing knowledge and emphasizes the need for well-designed studies to establish evidence-based guidelines for the use of vitamins in cardiovascular health management.

METHODOLOGY

Databases and Search Strategy

To conduct a comprehensive literature review on the role of vitamins in cardiovascular disease (CVD) prevention and management, multiple databases were searched, including PubMed, Scopus, Web of Science, Cochrane Library, and Google Scholar. These sources provided access to peer-reviewed studies, systematic reviews, meta-analyses, and grey literature.

Search Terms and Keywords

Relevant studies were identified using primary keywords such as "Vitamin C," "Vitamin D," "Vitamin E," "B-complex vitamins," "Vitamin K," "Cardiovascular Disease," "Prevention," and "Treatment." Secondary terms included "Oxidative Stress," "Atherosclerosis," "Hypertension," "Stroke," and "Endothelial Function." Example search strings included:

("Vitamin C" and "Cardiovascular Disease" and ("Prevention" or "Treatment")

("Vitamin D" and "Hypertension" and "Cardiovascular Risk")

Inclusion Criteria: Randomized controlled trials (RCTs), observational studies, systematic reviews, English-language articles (1993–present), and studies on adult populations at risk for or diagnosed with CVD.

Exclusion Criteria: Non-human studies, research on non-cardiovascular conditions, and studies lacking relevant cardiovascular data.

Data Extraction and Synthesis

Key outcomes included vitamin supplementation's impact on cardiovascular risk, oxidative stress, endothelial function, inflammation, myocardial infarction, stroke, and hypertension.

Data from clinical trials and meta-analyses were synthesized to highlight trends, mechanisms, and research gaps.

Risk of Bias and Study Selection

Biases such as selection, performance, detection, and funding biases were assessed. Mitigation strategies included randomization, blinding, and confounder control. Of 172 identified studies, 41 met inclusion criteria, contributing to a comprehensive analysis of vitamins' role in cardiovascular health.

Effect of Vitamin C on Cardiovascular Disease (CVD)

Vitamin C, also known as ascorbic acid, plays a vital role in reducing the risk of cardiovascular diseases (CVD). Its antioxidant properties, ability to improve endothelial function, and role in collagen synthesis contribute to its cardiovascular benefits.

Reduction of Oxidative Stress

Vitamin C neutralizes reactive oxygen species (ROS) and protects against oxidative damage to lipids, proteins, and DNA, which are implicated in atherosclerosis and other cardiovascular conditions (Carr & Maggini, 2017; Bhattacharjee *et al.,* 2024).

Improvement of Endothelial Function

By enhancing nitric oxide (NO) bioavailability, vitamin C improves endothelial-dependent vasodilation, reducing arterial stiffness and lowering blood pressure (Morelli *et al.,* 2020).

Anti-inflammatory Effects

Chronic inflammation is a significant contributor to atherosclerosis. Vitamin C reduces pro-inflammatory markers like C-reactive protein (CRP), thereby mitigating inflammation (Dhalla *et al.,* 2000).

*Lipid Profile Improvement***:**

Vitamin C may decrease LDL oxidation and improve HDL functionality, reducing the risk of plaque formation in arteries (Retsky *et al.,* 1993).

Collagen Synthesis

Adequate collagen synthesis is essential for maintaining the structural integrity of blood vessels. Vitamin C supports this process, reducing the risk of vessel rupture or aneurysm (Padayatty *et al.,* 2003).

Reduction in Hypertension

Vitamin C supplementation has been shown to lower blood pressure in hypertensive patients, reducing strain on the cardiovascular system (Juraschek *et al.,* 2012).

Mechanism of Action of Vitamin C in Cardiovascular Health

Antioxidant Activity

Vitamin C acts as a potent antioxidant by donating electrons to neutralize free radicals, thereby preventing oxidative stress, a critical factor in endothelial dysfunction and atherosclerosis (Frei, 1991; Carr & Maggini, 2017).

Enhancement of Nitric Oxide (NO) Availability

Vitamin C stabilizes NO by reducing superoxide anion-mediated NO degradation, leading to improved vasodilation and blood flow (Morelli *et al.,* 2020).

Inhibition of LDL Oxidation

By scavenging free radicals, vitamin C prevents the oxidative modification of LDL cholesterol, a crucial step in the initiation and progression of atherosclerosis (Retsky *et al.,* 1993).

Regulation of Endothelial Function

Vitamin C improves endothelial cell survival and reduces apoptosis, which is essential for maintaining vascular homeostasis (Ashor *et al.,* 2015).

Reduction of Inflammation

Vitamin C decreases inflammatory cytokines and markers like CRP, reducing vascular inflammation and slowing the progression of atherosclerosis (Dhalla *et al.,* 2000).

Collagen Formation

As a cofactor for prolyl and lysyl hydroxylase enzymes, vitamin C is critical for the synthesis of stable collagen, ensuring the strength and elasticity of blood vessel walls (Padayatty *et al.,* 2003).

Effect of Vitamin D on Cardiovascular Disease (CVD)

Vitamin D is crucial for maintaining cardiovascular health, with its deficiency linked to increased risk factors such as hypertension, atherosclerosis, and heart failure. Its effects are mediated through its regulation of calcium metabolism, anti-inflammatory properties, and impact on vascular function.

Regulation of Blood Pressure

Vitamin D reduces renin production, lowering the activity of the renin-angiotensin-aldosterone system (RAAS) and thereby helping regulate blood pressure (Li *et al.,* 2002).

Anti-inflammatory Effects

It suppresses pro-inflammatory cytokines and enhances anti-inflammatory mediators, reducing vascular inflammation and atherosclerosis (Norman & Powell, 2014).

Improvement of Endothelial Function

By promoting nitric oxide (NO) synthesis and reducing oxidative stress, vitamin D enhances endothelial function, which is critical for vascular health (Kim *et al.,* 2020).

Inhibition of Vascular Calcification

Vitamin D prevents vascular smooth muscle cell (VSMC) calcification by modulating calcium and phosphate metabolism, reducing arterial stiffness (Hou *et al.,* 2017).

Reduction in Insulin Resistance

Vitamin D improves insulin sensitivity, thereby mitigating the risk of type 2 diabetes—a major risk factor for CVD (Forouhi *et al.,* 2008).

Cholesterol Modulation

It has a role in reducing LDL cholesterol levels and increasing HDL, which can lower the risk of plaque formation in arteries (Pilz *et al.,* 2016).

Modulation of the RAAS

Vitamin D inhibits renin gene expression, leading to decreased RAAS activity, which helps in blood pressure regulation and reduces cardiac hypertrophy (Li *et al.,* 2002).

Regulation of Calcium and Phosphate Homeostasis

Vitamin D maintains calcium and phosphate balance, essential for vascular health, preventing arterial calcification (Hou *et al.,* 2017).

Anti-inflammatory Mechanisms

It suppresses NF-κB signaling, reducing the production of inflammatory cytokines such as IL-6 and TNF-α, which are implicated in atherosclerosis (Norman & Powell, 2014).

Endothelial Function Enhancement

Vitamin D promotes endothelial nitric oxide synthase (eNOS) activity, improving NO bioavailability and preventing endothelial dysfunction (Kim *et al.,* 2020).

Antioxidant Effects

By reducing oxidative stress and lipid peroxidation, vitamin D protects endothelial cells and prevents atherogenesis (Pilz *et al.,* 2016).

Impact on Lipid Metabolism

Vitamin D influences the expression of genes involved in lipid metabolism, reducing LDL oxidation and supporting a healthier lipid profile (Forouhi *et al.,* 2008).

Effect of Vitamin E on Cardiovascular Disease (CVD)

Vitamin E, a fat-soluble vitamin consisting of eight compounds (α-, β-, γ-, δ-tocopherols and tocotrienols), is well-known for its antioxidant properties. It protects cellular membranes and lipoproteins from oxidative damage, which is crucial in preventing cardiovascular diseases (CVDs) (Bhattacharjee & Pal, 2014).

Mechanisms of Action

Inhibition of LDL Oxidation: Vitamin E prevents the oxidation of low-density lipoproteins (LDL), a key step in atherosclerosis, thus reducing foam cell formation and arterial plaque buildup (Khatana *et al.,* 2020).

Anti-inflammatory Effects: Vitamin E decreases the expression of inflammatory markers such as IL-6 and C-reactive protein (CRP), both of which are elevated in atherosclerosis (Asbaghi *et al.,* 2020).

Improvement in Endothelial Function: Vitamin E enhances endothelial function by reducing oxidative stress and improving nitric oxide bioavailability, leading to better vascular relaxation (Su, 2015).

Platelet Aggregation Inhibition: Vitamin E inhibits platelet aggregation and adhesion, which are critical steps in thrombus formation and ischemic events like myocardial infarction (Steiner, 1999).

Clinical Evidence

Observational Studies: The Nurses' Health Study found that higher dietary vitamin E intake was inversely related to coronary heart disease risk (Khatana *et al.,* 2020).

Potential Risks and Recommendations

Excessive intake of vitamin E may lead to pro-oxidant effects, negating its antioxidant benefits and potentially resulting in adverse health outcomes. Current guidelines suggest obtaining vitamin E primarily from dietary sources, such as nuts, seeds, and vegetable oils, rather than high-dose supplements (Bhattacharjee & Pal, 2014).

Reduction of Oxidative Stress

Vitamin E protects cell membranes from oxidative damage by neutralizing free radicals,

reducing oxidative stress, which is a major factor in atherosclerosis (Bhattacharjee & Pal, 2014).

Prevention of LDL Oxidation

It inhibits the oxidation of low-density lipoproteins (LDL), preventing the formation of atherosclerotic plaques (Bhattacharjee & Pal, 2014).

Anti-inflammatory Effects

Vitamin E suppresses the production of pro-inflammatory cytokines like IL-6 and TNF-α, reducing vascular inflammation (Reiter *et al.,* 2007).

Improvement of Endothelial Function

By enhancing nitric oxide (NO) bioavailability and reducing endothelial oxidative stress, vitamin E improves endothelial function, crucial for vascular health (Roberts *et al.,* 2007).

Inhibition of Platelet Aggregation

Vitamin E reduces platelet aggregation and adhesion, lowering the risk of thrombosis and stroke (Steiner, 1999).

Reduction in Arterial Stiffness

Vitamin E supplementation improves arterial elasticity, reducing the risk of hypertension and vascular complications (Meydani *et al.,* 1997).

Mechanism of Action of Vitamin E in Cardiovascular Health

Antioxidant Activity

Vitamin E acts as a lipid-soluble antioxidant, incorporating into cellular membranes and scavenging lipid peroxyl radicals, thereby preventing lipid peroxidation and protecting against oxidative stress (Brigelius-Flohé & Traber, 1999).

Inhibition of LDL Oxidation

By donating hydrogen atoms to free radicals, vitamin E prevents the oxidative modification of LDL cholesterol, a crucial step in the initiation of atherosclerosis (Upston *et al.,* 2003).

Regulation of Inflammatory Pathways

Vitamin E downregulates the activity of NF-κB, reducing the expression of pro-inflammatory genes and cytokines (Reiter *et al.,* 2007).

Enhancement of Endothelial Nitric Oxide (NO) Availability

By reducing superoxide production, vitamin E increases NO bioavailability, promoting vasodilation and improving blood flow (Roberts *et al.,* 2007).

Inhibition of Platelet Aggregation

Vitamin E interferes with protein kinase C (PKC) activity, inhibiting platelet aggregation and reducing the risk of thrombus formation (Steiner, 1999).

Improvement in Vascular Elasticity

Vitamin E prevents the stiffening of arteries by reducing oxidative and inflammatory damage to vascular smooth muscle cells (Meydani *et al.,* 1997).

Effect of Vitamin B Complex on Cardiovascular Disease (CVD)

The Vitamin B complex, comprising several water-soluble vitamins, plays a crucial role in cardiovascular health. Deficiency in specific B vitamins, such as B6, B12, and folate, is linked to hyperhomocysteinemia, a significant risk factor for cardiovascular diseases. These vitamins also influence energy metabolism, reduce oxidative stress, and regulate inflammatory responses.

Reduction of Homocysteine Levels

Vitamins B6, B12, and folate lower homocysteine levels, a known risk factor for atherosclerosis and thrombosis (Bhattacharjee and Pal, 2014b, Mohan *et al.,* 2023).

Anti-inflammatory Effects

Vitamin B6 suppresses pro-inflammatory cytokines, reducing vascular inflammation and the risk of atherosclerosis (Friso *et al.,* 2001).

Improvement in Lipid Profile

Niacin (Vitamin B3) improves lipid metabolism by reducing LDL cholesterol, triglycerides, and lipoprotein(a) while increasing HDL cholesterol (Carlson, 2005).

Regulation of Energy Metabolism

Thiamine (Vitamin B1) enhances cardiac energy production by facilitating carbohydrate metabolism, improving heart function (Mrowicka *et al.,* 2023).

Prevention of Endothelial Dysfunction

Riboflavin (Vitamin B2) and niacin improve endothelial function by reducing oxidative stress and enhancing nitric oxide bioavailability (Mrowicka *et al.,* 2023).

Reduction in Hypertension Risk

Vitamin B2 supplementation reduces blood pressure, particularly in individuals with a specific genetic variant (MTHFR C677T polymorphism) (McNulty *et al.,* 2006).

Mechanism of Action of Vitamin B Complex in Cardiovascular Health

Homocysteine Metabolism

Vitamins B6, B12, and folate are co-factors in the remethylation and transsulfuration pathways of homocysteine metabolism, reducing plasma homocysteine levels and preventing endothelial damage (Mohan *et al.,* 2023).

Regulation of Lipid Metabolism

Niacin inhibits hepatic synthesis of VLDL and LDL cholesterol and enhances HDL levels, reducing the risk of plaque formation in arteries (Carlson, 2005).

Anti-inflammatory Mechanisms

Vitamin B6 inhibits the NF-κB pathway, reducing the production of pro-inflammatory cytokines like IL-6 and TNF-α, protecting against vascular inflammation (Friso *et al.,* 2001).

Oxidative Stress Reduction

Riboflavin and niacin act as coenzymes in redox reactions, reducing oxidative damage to endothelial cells and improving vascular function (Mrowicka *et al.,* 2023).

Improvement of Myocardial Energy Metabolism

Thiamine facilitates the conversion of pyruvate to acetyl-CoA, ensuring adequate ATP production in cardiac cells, critical for heart function (Mrowicka *et al.,* 2023).

Blood Pressure Modulation

Riboflavin enhances the metabolism of nitric oxide, improving vasodilation and reducing blood pressure, especially in genetically susceptible individuals (McNulty *et al.,* 2006).

Overall Mechanism

The mechanism of action of vitamins in cardiovascular health encompasses multiple pathways vital for preventing and managing cardiovascular diseases (CVDs). Vitamin C, a potent antioxidant, neutralizes reactive oxygen species (ROS) to mitigate oxidative stress, improves nitric oxide (NO) bioavailability for endothelial function, reduces pro-inflammatory markers, and supports collagen synthesis for vascular integrity (Carr & Maggini, 2017; Padayatty *et al.,* 2003). Vitamin D regulates calcium-phosphate balance, inhibits vascular calcification, suppresses inflammation, modulates the renin-angiotensin-aldosterone system (RAAS), and enhances endothelial NO availability, reducing hypertension and atherosclerosis risks (Li *et al.,* 2002; Norman & Powell, 2014). Vitamin E, a lipid-soluble antioxidant, prevents LDL oxidation, reduces inflammatory cytokines, inhibits platelet aggregation, and promotes vascular elasticity through its antioxidant and anti-inflammatory effects (Brigelius-Flohé & Traber, 1999; Reiter *et al.,* 2007). The B-complex vitamins lower homocysteine, an atherogenic marker, improve lipid profiles through niacin, regulate energy metabolism via thiamine, and enhance endothelial function by reducing oxidative stress and promoting NO bioavailability (Mohan *et al.,* 2023; Carlson, 2005). Collectively, these vitamins act through antioxidative, anti-inflammatory, lipid-regulating, and vasodilatory mechanisms to reduce the risk of CVDs while maintaining vascular health.

CONCLUSION

The review concludes that vitamins play a critical role in cardiovascular disease (CVD) prevention and management by modulating key physiological processes. Vitamins C, D, E, B complex, and K exhibit antioxidative, anti-inflammatory, and lipid-regulating properties that enhance vascular health, reduce oxidative stress, and prevent atherosclerosis. While promising evidence supports their potential, variability in outcomes due to study design, dosage, and patient population underscores the need for further research. Excessive supplementation, particularly of fat-soluble vitamins, poses risks, highlighting the importance of balanced intake through diet and supplements. This review emphasizes integrating vitamins as complementary agents in CVD care and the necessity for well-designed trials to establish definitive guidelines for their optimal use in improving cardiovascular health.

REFERENCES

1. Asbaghi O, Sadeghian M, Nazarian B, Sarreshtedari M, Mozaffari-Khosravi H, Maleki V, Alizadeh M, Shokri A, Sadeghi O. The effect of vitamin E supplementation on selected inflammatory biomarkers in adults: a systematic review and meta-analysis of randomized clinical trials. Sci Rep. 2020;10(1):17234. https://doi.org/10.1038/s41598-020-73741-6
2. Ashor AW, Siervo M, Lara J, Oggioni C, Afshar S, Mathers JC. Effect of vitamin C and vitamin E supplementation on endothelial function: a systematic review and meta-analysis of randomised controlled trials. Br J Nutr.

2015;113(8):1182-94. https://doi.org/10.1017/S0007114515000227

3. Bhattacharjee S, Pal S. Additive protective effects of selenium and vitamin E against arsenic induced lipidemic and cardiotoxic effects in mice. IJPPS. 2014a;6(5):406-413.
4. Bhattacharjee S, Pal S. Antilipidemic and cardioprotective effects of vitamin B_{12} and folic acid against arsenic toxicity. IJPCBS. 2014b;4(2):353-360.
5. Bhattacharjee S, Chakraborty S, Sharma A, Chakraborty A, Das PK, Das P, Lodh D, Chakraborty TR. Molecular Insights into the Interaction of Vitamin C (Ascorbic Acid) with Glutathione Peroxidase: A Comprehensive Computational Study. Med & Analy Chem Int J. 2024;8(1):000188. DOI: 10.23880/macij-16000188
6. Brigelius-Flohé R, Traber MG. Vitamin E: function and metabolism. FASEB J. 1999;13(10):1145-55. https://doi.org/10.1096/fasebj.13.10.1145
7. Carlson LA. Nicotinic acid: the broad-spectrum lipid drug. A 50th anniversary review. J Intern Med. 2005;258(2):94-114. https://doi.org/10.1111/j.1365-2796.2005.01528.x
8. Carr AC, Maggini S. Vitamin C and Immune Function. Nutrients. 2017; 9(11):1211. https://doi.org/10.3390/nu9111211
9. Catalgol B, Ozer NK. Protective effects of vitamin E against hypercholesterolemia-induced age-related diseases. Genes Nutr. 2012;7(1):91-8. https://doi.org/10.1007/s12263-011-0235-9
10. de la Guía-Galipienso F, Martínez-Ferran M, Vallecillo N, Lavie CJ, Sanchis-Gomar F, Pareja-Galeano H. Vitamin D and cardiovascular health. Clin Nutr. 2021;40(5):2946-2957. https://doi.org/10.1016/j.clnu.2020.12.025
11. Dhalla NS, Temsah RM, Netticadan T. Role of oxidative stress in cardiovascular diseases. J Hypertens. 2000;18(6):655-73. https://doi.org/10.1097/00004872-200018060-00002
12. Di Cesare M, Perel P, Taylor S, Kabudula C, Bixby H, Gaziano TA, McGhie DV, Mwangi J, Pervan B, Narula J, Pineiro D, Pinto FJ. The Heart of the World. Glob Heart. 2024;19(1):11. https://doi.org/10.5334/gh.1288
13. Forouhi N.G., Luan J, Cooper A, Boucher BJ, Wareham NJ. Baseline serum 25-hydroxy vitamin d is predictive of future glycemic status and insulin resistance: the Medical Research Council Ely Prospective Study 1990-2000. Diabetes. 2008; 57(10):2619-25. https://doi.org/10.2337/db08-0593
14. Friso S, Jacques PF, Wilson PW, Rosenberg IH, Selhub J. Low circulating vitamin B(6) is associated with elevation of the inflammation marker C-reactive protein independently of plasma homocysteine levels. Circulation. 2001;103(23):2788-91. https://doi.org/10.1161/01.cir.103.23.2788
15. Hou YC, Liu WC, Zheng CM, Zheng JQ, Yen TH, Lu KC. Role of Vitamin D in Uremic Vascular Calcification. Biomed Res Int. 2017;2017:2803579. https://doi.org/10.1155/2017/2803579
16. Juraschek SP, Guallar E, Appel LJ, Miller ER 3rd. Effects of vitamin C supplementation on blood pressure: a meta-analysis of randomized controlled trials. Am J Clin Nutr. 2012;95(5):1079-88. https://doi.org/10.3945/ajcn.111.027995
17. Khatana C, Saini NK, Chakrabarti S, Saini V, Sharma A, Saini RV, Saini AK. Mechanistic Insights into the Oxidized Low-Density Lipoprotein-Induced Atherosclerosis. Oxid Med Cell Longev. 2020;2020:5245308. https://doi.org/10.1155/2020/5245308
18. Kim DH, Meza CA, Clarke H, Kim JS, Hickner RC. Vitamin D and Endothelial Function. Nutrients. 2020; 12(2):575. https://doi.org/10.3390/nu12020575
19. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. J Clin Invest. 2002;110(2):229-38. https://doi.org/10.1172/JCI15219
20. Marchioli R, Schweiger C, Levantesi G, Tavazzi L, Valagussa F. Antioxidant vitamins and prevention of cardiovascular disease: epidemiological and clinical trial data. Lipids. 2001;36 Suppl:S53-63. https://doi.org/10.1007/s11745-001-0683-y
21. May JM, Harrison FE. Role of vitamin C in the function of the vascular endothelium. Antioxid Redox Signal. 2013;19(17):2068-83.https://doi.org/10.1089/ars.2013.5205
22. McNulty H, Dowey le RC, Strain JJ, Dunne A, Ward M, Molloy AM, McAnena LB, Hughes JP, Hannon-Fletcher M, Scott JM. Riboflavin lowers homocysteine in individuals homozygous for the MTHFR 677C->T polymorphism. Circulation. 2006;113(1):74-80. https://doi.org/10.1161/Circulationaha.105.580332
23. Meydani SN, Meydani M, Blumberg JB, Leka LS, Siber G, Loszewski R, Thompson C, Pedrosa MC, Diamond RD, Stollar BD. Vitamin E supplementation and in vivo immune response in healthy elderly subjects. A randomized controlled trial. JAMA. 1997; 277(17):1380-6. https://doi.org/10.1001/jama.1997.03540410058031
24. Mohan A, Kumar R, Kumar V, Yadav M. Homocysteine, Vitamin B12 and Folate Level: Possible Risk Factors in the Progression of Chronic Heart and Kidney Disorders. Curr Cardiol Rev. 2023;19(4):e090223213539. https://doi.org/10.2174/1573403X19666230209111854
25. Morelli MB, Gambardella J, Castellanos V, Trimarco V, Santulli G. Vitamin C and Cardiovascular Disease: An Update. Antioxidants (Basel). 2020;9(12):1227.https://doi.org/10.3390/antiox9121227
26. Mrowicka M, Mrowicki J, Dragan G, Majsterek I. The importance of thiamine (vitamin B1) in

humans. Biosci Rep. 2023; 43(10):BSR20230374. https://doi.org/10.1042/BSR20230374

27. Münzel T, Camici GG, Maack C, Bonetti NR, Fuster V, Kovacic JC. Impact of Oxidative Stress on the Heart and Vasculature: Part 2 of a 3-Part Series. J Am Coll Cardiol. 2017; 70(2):212-229. https://doi.org/10.1016/j.jacc.2017.05.035
28. Nardin M, Verdoia M, Nardin S, Cao D, Chiarito M, Kedhi E, Galasso G, Condorelli G, De Luca G. Vitamin D and Cardiovascular Diseases: From Physiology to Pathophysiology and Outcomes. Biomedicines. 2024; 12(4):768. https://doi.org/10.3390/biomedicines12040768
29. Norman PE, Powell JT. Vitamin D and cardiovascular disease. Circ Res. 2014; 114(2):379-93. https://doi.org/10.1161/CIRCRESAHA.113.301241
30. Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee JH, Chen S, Corpe C, Dutta A, Dutta SK, Levine M. Vitamin C as an antioxidant: evaluation of its role in disease prevention. J Am Coll Nutr. 2003; 22(1):18-35. https://doi.org/10.1080/07315724.2003.10719272
31. Pilz S, Verheyen N, Grübler MR, Tomaschitz A, März W. Vitamin D and cardiovascular disease prevention. Nat Rev Cardiol. 2016;13(7):404-17. https://doi.org/10.1038/nrcardio.2016.73
32. Reddy P, Jialal I. Biochemistry, Fat Soluble Vitamins. [Updated 2022 Sep 19]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK534869/
33. Reiter E, Jiang Q, Christen S. Anti-inflammatory properties of alpha- and gamma-tocopherol. Mol Aspects Med. 2007; 28(5-6):668-91. https://doi.org/10.1016/j.mam.2007.01.003
34. Retsky KL, Freeman MW, Frei B. Ascorbic acid oxidation product(s) protect human low density lipoprotein against atherogenic modification. Anti- rather than prooxidant activity of vitamin C in the presence of transition metal ions. J Biol Chem. 1993; 268(2):1304-9
35. Roberts LJ 2nd, Oates JA, Linton MF, Fazio S, Meador BP, Gross MD, Shyr Y, Morrow JD. The relationship between dose of vitamin E and suppression of oxidative stress in humans. Free Radic Biol Med. 2007; 43(10):1388-93.https://doi.org/10.1016/j.freeradbiomed.2007.06.019
36. Simsek B, Selte A, Egeli BH, Çakatay U. Effects of vitamin supplements on clinical cardiovascular outcomes: Time to move on! - A comprehensive review. Clin Nutr ESPEN. 2021; 42:1-14. https://doi.org/10.1016/j.clnesp.2021.02.014
37. Steiner M. (1999). Vitamin E, a modifier of platelet function: rationale and use in cardiovascular and cerebrovascular disease. Nut. Rev. 1999; 57(10):306–309. https://doi.org/10.1111/j.1753-4887.1999.tb06903.x
38. Su JB. Vascular endothelial dysfunction and pharmacological treatment. World J Cardiol. 2015; 7(11):719-41.https://doi.org/10.4330/wjc.v7.i11.719
39. Upston JM, Kritharides L, Stocker R. The role of vitamin E in atherosclerosis. Prog Lipid Res. 2003; 42(5):405-22. https://doi.org/10.1016/s0163-7827(03)00024-9
40. van Gorp RH, Baaten CCFMJ, Habibi A, Jaminon AMG, Peeters FECM, Leenders P, Crijns HJGMC, Heemskerk JWM, Reutelingsperger CP, Spronk HM, Schurgers LJ. Vitamin K antagonist use induces calcification and atherosclerotic plaque progression resulting in increased hypercoagulability. Eur Heart J Open. 2021; 1(2):oeab017. https://doi.org/10.1093/ehjopen/oeab017

Letter to Editor

International Physiology
Volume 12 Number 3-4, May - December 2024
DOI: https://dx.doi.org/10.21088/ip.2347.1506.122234.4

A Call for Commitment to Scholarly Ethics in Medical Research and Education

Prerna Agarwal

Assistant Professor, Department of Physiology, Government Institute of Medical Sciences, Greater Noida 201310, Uttar Pradesh, India.

E-mail: dr.preranaagarwal@gmail.com

Abstract

Ethics form the cornerstone of academic work, guiding individuals in their pursuit of knowledge, truth, and intellectual growth. They emphasize honesty, integrity, and ethical conduct in all academic endeavors, fostering a culture of trust and respect. However, there have been many retractions of published papers from journals of great repute in recent years. Such incidents underscore the importance of scholarly ethics in medical education and research. As educators and researchers, we bear the responsibility of modelling ethical standards, which form the foundation of trust in academia and healthcare.

Keywords: Ethics, Research; Plagiarism; Retraction of Publication; Biomedical Research.

Recently, there have been many retractions of published papers from journals of great repute. Such incidents underscore the importance of scholarly ethics in medical education and research.[1,2] Ethics form the cornerstone of academic work, guiding individuals in their pursuit of knowledge, truth, and intellectual growth. They emphasize honesty, integrity, and ethical conduct in all academic endeavors, fostering a culture of trust and respect. Honesty is especially central to scholarly ethics, requiring accurate and transparent representation of ideas and research findings. The growing pressure to publish has notably increased unethical practices among students, faculty, and researchers. Practices such as plagiarism, data manipulation, and ghost authorship not only compromise academic integrity but also erode trust within the community. Plagiarism—the use of others' ideas or work without proper attribution—seriously undermines academic integrity and erodes trust within the academic community. Similarly, while collaboration is vital for exchanging ideas, it is essential to distinguish it from cheating, ensuring fair contributions and proper acknowledgment. Failing to do so compromises individual and institutional credibility and undermines the integrity of medical science, which can ultimately impact patient care. Another unethical practice that has increasingly plagued academia is the submission of fake peer reviews using the names of preferred reviewers, further compromising the integrity of the scholarly process.[3, 4, 5]

As educators and researchers, we bear the responsibility of modelling ethical standards, which form the foundation of trust in academia and healthcare. Researchers must conduct studies ethically, protecting participants' rights and ensuring the validity and reliability of findings. Equally important is upholding ethical principles in authorship and publication, ensuring that contributions are fairly represented, credit is properly assigned, and the highest standards of integrity are maintained in research reporting.

To address challenges to scholarly ethics, higher education institutions should establish clear guidelines and promote academic honesty through integrity policies, workshops, and rigorous peer-review processes. Faculty must lead by example, mentoring students on transparency, honesty, and accountability.I also advocate for academic journals to maintain stringent review and plagiarism detection processes and encourage authors to adhere to the highest ethical standards. By upholding these

This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0.

principles, we can create an environment where academic excellence is achieved through merit and integrity. Our commitment to scholarly ethics will go a long way in the advancement of healthcare research.

REFERENCES

1. Khademizadeh S, Danesh F, Esmaeili S, Lund B, Santos-d'Amorim K. Evolution of retracted publications in the medical sciences: Citations analysis, bibliometrics, and altmetrics trends. Account Res. 2023; 1–16. https://doi.org/10.1080/08989621.2023.2223996
2. Brundy C, Thornton JB. The paper mill crisis is a five-alarm fire for science: what can librarians do about it? Insights UKSG J. 2024; 37(1):11. https://doi.org/10.1629/uksg.659
3. Bain LE, Ebuenyi ID, Noubiap JJ. Research and publication ethics knowledge and practices in the health and life sciences: Findings from an exploratory survey. Ethics Med Public Health. 2022; 23:100803. https://doi.org/10.1016/j.jemep.2022.100803
4. Pan P. Ethics in research and publication. J Indian Assoc Pediatr Surg. 2020;25(6):349–51. https://doi.org/10.4103/jiaps.JIAPS_219_19
5. Rivera H, Teixeira da Silva JA. Retractions, fake peer reviews, and paper mills. J Korean Med Sci. 2021; 36(24).https://doi.org/10.3346/jkms.2021.36.e165

How to cite this article:

Prerna Agarwal, A Call for Commitment to Scholarly Ethics in Medical Research and Education. Int.Phy.2024;12(2-3):64-65.

Subject Index

Author Index

Guidelines for Authors

Manuscripts must be prepared in accordance with "Uniform requirements for Manuscripts submitted to Biomedical Journal" developed by international committee of medical Journal Editors

Types of Manuscripts and Limits

Original articles: Up to 3000 words excluding references and abstract and up to 10 references.

Review articles: Up to 2500 words excluding references and abstract and up to 10 references.

Case reports: Up to 1000 words excluding references and abstract and up to 10 references.

Online Submission of the Manuscripts

Articles can also be submitted online from http://rfppl.co.in/customer_index.php.

I) First Page File: Prepare the title page, covering letter, acknowledgement, etc. using a word processor program. All information which can reveal your identity should be here. use text/rtf/doc/PDF files. Do not zip the files.

2) Article file: The main text of the article, beginning from Abstract till References (including tables) should be in this file. Do not include any information (such as acknowledgement, your name in page headers, etc.) in this file. Use text/rtf/doc/PDF files. Do not zip the files. Limit the file size to 400 Kb. Do not incorporate images in the file. If file size is large, graphs can be submitted as images separately without incorporating them in the article file to reduce the size of the file.

3) Images: Submit good quality color images. Each image should be less than 100 Kb in size. Size of the image can be reduced by decreasing the actual height and width of the images (keep up to 400 pixels or 3 inches). All image formats (jpeg, tiff, gif, bmp, png, eps etc.) are acceptable; jpeg is most suitable.

Legends: Legends for the Fig.s/images should be included at the end of the article file.

If the manuscript is submitted online, the contributors' form and copyright transfer form has to be submitted in original with the signatures of all the contributors within two weeks from submission. Hard copies of the images (3 sets), for articles submitted online, should be sent to the journal office at the time of submission of a revised manuscript. Editorial office: Red Flower Publication Pvt. Ltd., 48/41-42, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi – 110 091, India, Phone: 91-11-79695648, Cell: +91-9821671871. E-mail: author@rfppl.co.in. Submission page: http://rfppl.co.in/article_submission_system.php?mid=5.

Preparation of the Manuscript

The text of observational and experimental articles should be divided into sections with the headings: Introduction, Methods, Results, Discussion, References, Tables, Fig.s, Fig. legends, and Acknowledgment. Do not make subheadings in these sections.

Title Page

The title page should carry

1) Type of manuscript (e.g. Original article, Review article, Case Report)
2) The title of the article should be concise and informative;
3) Running title or short title not more than 50 characters;
4) The name by which each contributor is known (Last name, First name and initials of middle name), with his or her highest academic degree(s) and institutional affiliation;
5) The name of the department(s) and institution(s) to which the work should be attributed;
6) The name, address, phone numbers, facsimile numbers and e-mail address of the contributor responsible for correspondence about the manuscript; should be mentoined.
7) The total number of pages, total number of photographs and word counts separately for abstract and for the text (excluding the references and abstract);
8) Source(s) of support in the form of grants, equipment, drugs, or all of these;
9) Acknowledgement, if any; and
l0) If the manuscript was presented as part at a meeting, the organization, place, and exact date on which it was read.

Abstract Page

The second page should carry the full title of the manuscript and an abstract (of no more than 150 words for case reports, brief reports and 250 words for original articles). The abstract should be structured and state the Context (Background), Aims, Settings and Design, Methods and Materials, Statistical Analyzis used, Results and Conclusions. Below the abstract should provide 3 to 10 keywords.

Introduction

State the background of the study and purpose of the study and summarize the rationale for the study or observation.

Methods

The methods section should include only information that was available at the time the plan or protocol for the study was written such as study approach, design, type of sample, sample size, sampling technique, setting of the study, description of data collection tools and methods; all information obtained during the conduct of the study belongs in the Results section.

Reports of randomized clinical trials should be based on the CONSORT Statement (http:// www. consort-statement. org). When reporting experiments on human subjects, indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000 (available at http://www.wma. net/e/policy/l7-c_e.html).

Results

Present your results in logical sequence in the text, tables, and illustrations, giving the main or most important findings first. Do not repeat in the text all the data in the tables or illustrations; emphasize or summarize only important observations. Extra or supplementary materials and technical details can be placed in an appendix where it will be accessible but will not interrupt the flow of the text; alternatively, it can be published only in the electronic version of the journal.

Discussion

Include summary of key findings (primary outcome measures, secondary outcome measures, results as they relate to a prior hypothesis); Strengths and limitations of the study (study question, study design, data collection, Analyzis and interpretation); Interpretation and implications in the context of the totality of evidence (is there a systematic review to refer to, if not, could one be reasonably done here and now?, What this study adds to the available evidence, effects on patient care and health policy, possible mechanisms)? Controversies raised by this study; and Future research directions (for this particular research collaboration, underlying mechanisms, clinical research). Do not repeat in detail data or other material given in the Introduction or the Results section.

References

List references in alphabetical order. Each listed reference should be cited in text (not in alphabetic order), and each text citation should be listed in the References section. Identify references in text, tables, and legends by Arabic numerals in square bracket (e.g. [10]). Please refer to ICMJE Guidelines (http://www.nlm.nih.gov/bsd/uniform_ requirements.html) for more examples.

Standard journal article

[1] Flink H, Tegelberg Å, Thörn M, Lagerlöf F. Effect of oral iron supplementation on unstimulated salivary flow rate: A randomized, double-blind, placebo-controlled trial. J Oral Pathol Med 2006; 35: 540–7.

[2] Twetman S, Axelsson S, Dahlgren H, Holm AK, Källestål C, Lagerlöf F, et al. Caries-preventive effect of fluoride toothpaste: A systematic review. Acta Odontol Scand 2003; 61: 347–55.

Article in supplement or special issue

[3] Fleischer W, Reimer K. Povidone-iodine antisepsis. State of the art. Dermatology 1997; 195 Suppl 2: 3–9.

Corporate (collective) author

[4] American Academy of Periodontology. Sonic and ultrasonic scalers in periodontics. J Periodontol 2000; 71: 1792–801.

Unpublished article

[5] Garoushi S, Lassila LV, Tezvergil A, Vallittu PK. Static and fatigue compression test for particulate filler composite resin with fiber-reinforced composite substructure. Dent Mater 2006.

Personal author(s)

[6] Hosmer D, Lemeshow S. Applied logistic regression, 2nd edn. New York: Wiley-Interscience; 2000.

Chapter in book

[7] Nauntofte B, Tenovuo J, Lagerlöf F. Secretion

and composition of saliva. In: Fejerskov O,

Kidd EAM, editors. Dental caries: The disease and its clinical management. Oxford: Blackwell Munksgaard; 2003. pp 7–27.

No author given

[8] World Health Organization. Oral health surveys–basic methods, 4th edn. Geneva: World Health Organization; 1997.

Reference from electronic media

[9] National Statistics Online—Trends in suicide by method in England and Wales, 1979–2001. www.statistics.gov.uk/downloads/theme_health/HSQ 20.pdf (accessed Jan 24, 2005): 7–18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.

More information about other reference types is available at www.nlm.nih.gov/bsd/uniform_ requirements.html, but observes some minor deviations (no full stop after journal title, no issue or date after volume, etc.).

Tables

Tables should be self-explanatory and should not duplicate textual material.

Tables with more than 10 columns and 25 rows are not acceptable.

Table numbers should be in Arabic numerals, consecutively in the order of their first citation in the text and supply a brief title for each.

Explain in footnotes all non-standard abbreviations that are used in each table.

For footnotes use the following symbols, in this sequence: *, ¶, †, ‡‡,

Illustrations (Fig.s)

Graphics files are welcome if supplied as Tiff, EPS, or PowerPoint files of minimum 1200x1600 pixel size. The minimum line weight for line art is 0.5 point for optimal printing.

When possible, please place symbol legends below the Fig. instead of the side.

Original color Fig.s can be printed in color at the editor's and publisher's discretion provided the author agrees to pay.

Type or print out legends (maximum 40 words, excluding the credit line) for illustrations using double spacing, with Arabic numerals corresponding to the illustrations.

Sending a revised manuscript

While submitting a revised manuscript, contributors are requested to include, along with single copy of the final revised manuscript, a photocopy of the revised manuscript with the changes underlined in red and copy of the comments with the point-to-point clarification to each comment. The manuscript number should be written on each of these documents. If the manuscript is submitted online, the contributors' form and copyright transfer form has to be submitted in original with the signatures of all the contributors within two weeks of submission. Hard copies of images should be sent to the office of the journal. There is no need to send printed manuscript for articles submitted online.

Reprints

Journal provides no free printed, reprints, however an author copy is sent to the main author and additional copies are available on payment (ask to the journal office).

Copyrights

The whole of the literary matter in the journal is copyright and cannot be reproduced without the written permission.

Declaration

A declaration should be submitted stating that the manuscript represents valid work and that neither this manuscript nor one with substantially similar content under the present authorship has been published or is being considered for publication elsewhere and the authorship of this article will not be contested by any one whose name(s) is/are not listed here, and that the order of authorship as placed in the manuscript is final and accepted by the co-authors. Declarations should be signed by all the authors in the order in which they are mentioned in the original manuscript. Matters appearing in the Journal are covered by copyright but no objection will be made to their reproduction provided permission is obtained from the Editor prior to publication and due acknowledgment of

the source is made.

Approval of Ethics Committee

We need the Ethics committee approval letter from an Institutional ethical committee (IEC) or an institutional review board (IRB) to publish your Research article or author should submit a statement that the study does not require ethics approval along with evidence. The evidence could either be consent from patients is available and there are no ethics issues in the paper or a letter from an IRB stating that the study in question does not require ethics approval.

Abbreviations

Standard abbreviations should be used and be spelt out when first used in the text. Abbreviations should not be used in the title or abstract.

Checklist

- Manuscript Title
- Covering letter: Signed by all contributors
- Previous publication/ presentations mentioned, Source of funding mentioned
- Conflicts of interest disclosed

Authors

- Middle name initials provided.
- Author for correspondence, with e-mail address provided.
- Number of contributors restricted as per the instructions.
- Identity not revealed in paper except title page (e.g. name of the institute in Methods, citing previous study as 'our study')

Presentation and Format

- Double spacing
- Margins 2.5 cm from all four sides
- Title page contains all the desired information. Running title provided (not more than 50 characters)
- Abstract page contains the full title of the manuscript
- Abstract provided: Structured abstract provided for an original article.
- Keywords provided (three or more)
- Introduction of 75–100 words
- Headings in title case (not ALL CAPITALS). References cited in square brackets
- References according to the journal's instructions

Language and grammar

- Uniformly American English
- Abbreviations spelt out in full for the first time. Numerals from 1 to l0 spelt out
- Numerals at the beginning of the sentence spelt out

Tables and Fig.s

- No repetition of data in tables and graphs and in text.
- Actual numbers from which graphs drawn, provided.
- Fig.s necessary and of good quality (color)
- Table and Fig. numbers in Arabic letters (not Roman).
- Labels pasted on back of the photographs (no names written)
- Fig. legends provided (not more than 40 words)
- Patients' privacy maintained, (if not permission taken)
- Credit note for borrowed Fig.s/tables provided
- Manuscript provided on a CDROM (with double spacing)

Submitting the Manuscript

- Is the journal editor's contact information current?
- Is the cover letter included with the manuscript? Does the letter:

1. Include the author's postal address, e-mail address, telephone number, and fax number for future correspondence?
2. State that the manuscript is original, not previously published, and not under concurrent consideration elsewhere?
3. Inform the journal editor of the existence of any similar published manuscripts written by the author?
4. Mention any supplemental material you are submitting for the online version of your article. Contributors' Form (to be modified as applicable and one signed copy attached with the manuscript)

Instructions to Authors

Submission to the journal must comply with the Guidelines for Authors.
Non-compliant submission will be returned to the author for correction.

To access the online submission system and for the most up-to-date version of the Guide for Authors please visit: http://www.rfppl.co.in

Technical problems or general questions on publishing with **IP** are supported by Red Flower Publication Pvt. Ltd.'s Author Support team (http://rfppl.co.in/article_submission_system.php?mid=5#)

Alternatively, please contact the Journal's Editorial Office for further assistance.

Editorial Manager
Red Flower Publication Pvt. Ltd.
48/41-42, DSIDC, Pocket-II
Mayur Vihar Phase-I
Delhi - 110 091(India)
Mobile: 9821671871, Phone: 91-11-79695648
E-mail: author@rfppl.co.in