PACIFIC JOURNAL OF MEDICAL SCIENCES

VOLUME 27, No 1, December 2025

PACIFIC JOURNAL OF MEDICAL SCIENCES

{Formerly: Medical Sciences Bulletin}

ISSN: 2072 – 1625

Pac. J. Med. Sci. (PJMS)

www.pacjmedsci1625.com. Email: managingeditorpjms1625@gmail.com.

ISSN: 2072 – 1625 Volume 27, No. 1, December 2025

A multidisciplinary journal for publication of medical and biomedical research findings on issues pertinent to improving family health and related issues of public health.

INFORMATION:
Global Index Medicus (GIM) new platform
Dear colleagues, WPRIM NJSC and focal persons,
The new GIM portal was launched by the WHO Library on October 31, 2019. It provides improved features, increasing better search results. GIM allows federated search in the five WHO regional indices including WPRIM. You can download the poster from the GIM website and share it with your other colleagues. For more information and other promotional materials, GIM website can be accessed at www.globalindexmedicus.net. Please help to encourage and maximize the use of GIM and WPRIM.

Western Pacific Region Index Medicus (WPRO)
The Western Pacific Region Index Medicus (WPRIM) is the regional bibliographic index of medical and health journals published by the Member States of the Western Pacific Region. This is the Region's contribution to the Global Index Medicus (GIM) initiative which aims to extend to all the benefits of the knowledge that is essential to the fullest attainment of health. WPRIM allows free public access to research and publications that may not be available through other popular medical indexes.

The website of the Pacific Journal of Medical Sciences (PJMS; Pac J Med Sci) is still in the process of migrating to a new platform. Please take note of the new website address: www.pacjmedsci1625.com
The migration of the archives is completed.

PACIFIC JOURNAL OF MEDICAL SCIENCES

(Formerly Medical Sciences Bulletin)

ISSN: 2072 – 1625 **Volume 27, No. 1, December 2025**

A multidisciplinary journal for publication of medical and biomedical research findings on issues pertinent to improving family health and related issues of public health

Editor – in – Chief

Associate Professor Phillip Kigodi

Associate Editors

Associate Professor Andrew Masta

Professor Francis Hombhanje

Managing Editors

Professor Victor J. Temple (Member of APAME)

Professor Lohi Matainaho

Specialty Editors and Editorial Board Members:

Dr. Nancy Buasi, Associate Prof. Jackson K. Lauwo, Dr. Wangi Linjim, Mr. Gairo Gerega, Dr. Paulus Ripa, Dr. M. Siddiqi, Prof. Z. S. C. Okoye, Dr. David K. Obatomi, Prof. B. O. Ogunbanjo, Prof. C. E. Anyiwo, Prof. Alphonsus N. Onyiriuka, Associate Prof. Florence Muga,

INFORMATION

SUBSCRIPTIONS: Correspondences concerning subscriptions, purchase of single copies and back issues, lost copies and related business should be addressed to the Secretary, Basic Medical Sciences, School of Medicine and Health Sciences, University of Papua New Guinea, P. O. Box 5623 Boroko, N.C.D., PNG. Official website is www.pacjmedsci1625.com; For Instructions to Authors please visit the official website.

December 2025: ISSN: 2072 – 1625: VOLUME 27, No. 1

TABLE OF CONTENTS | Page #

EDITORIAL

IS ADEQUATE NUTRITION THE BEST FORM OF PREVENTIVE MEDICINE?

Managing Editor Pacific Journal of Medical Sciences

Correspondence: managingeditorpjms1625@gmail.com

According to the World Health Organization (WHO), the general concept of adequate nutrition refers to the intake of essential micronutrients (vitamins and trace elements) and macronutrients (carbohydrate, protein and fat) in the appropriate quantities required to sustain metabolic and physiological processes and prevent disease. This concept is context dependent, because what is adequate for a group of individuals may be insufficient or excessive for another group.

In the present context, the concept of preventive medicine encompasses interventions designed to avert disease before its onset with the aim of reducing morbidity and mortality. Ultimately, these interventions increase economic productivity and have a positive impact on public health care. In a wider context, preventive medicine focuses on the health of individuals, communities, and specific populations to promote the health and well-being of individuals by preventing disease, disability and untimely death.

The idea that nutrition and preventive medicine are closely interrelated is widely attributed to the "father of modern medicine" Hippocrates of Cos (5th to 4th century BC) who is known for saying: "*Let food be your best medicine and your best medicine be your food*." This idea directly linked the concept of nutrition and health, based on closely observing the environmental causes of illness, including more general aspects of patient's life and their influence on their health and convalescence.

Current scientific evidence supports Hippocrates' ideas. According to the Americal College of Preventive Medicine, three levels of prevention can be identified:

Primary level – Preventing disease before it occurs. The patient is at risk for a disease but not yet affected. This level identifies behavioral, environmental, genetic and other factors that increase the chance of contracting the disease. Some of the risk factors, excluding genetic factors, can be changed. This level requires good health promotion, such as health

education, immunization, and correction of poor habits.

Secondary level – Detecting disease early to halt progression (e.g., through screenings). Risk factors can combine to cause a disease. It is usually unmanifested, clinically undetectable, but becoming detectable once specific pathological changes occur. For example, large amounts of blood in the stool are a warning sign of colorectal cancer. During pathogenesis, secondary prevention may be achieved by early diagnosis and prompt treatment.

Tertiary level – Managing established disease to prevent complications, when disease signs and symptoms appear. The clinical horizon is the point at which the condition can be scientifically detected. Most preventive health care focuses on this level. This level is the most expensive, in terms of the cost of health care.

Of these three levels, nutrition primarily falls under primary level, though it also influences secondary and tertiary levels by improving recovery and reducing complications. It is important to note that adequate nutrition offers a holistic approach compared to vaccinations and screenings that target specific diseases. Adequate nutrition also influences multiple systems simultaneously, such as cardiovascular system, metabolic system, immune system, and the nervous system, including mental health.

Current scientific evidence clearly indicates that adequate nutrition as a preventive measure is important across the entire lifespan of an individual:

During prenatal and early childhood: Fetal development, birth outcomes, and long-term health and risk of disease are influenced by the nutritional status of the mother. Breastfeeding and early dietary patterns shape the immune system and metabolic status of the infant. For example, maternal deficiency of iodine, folate, iron, and B12 severely affects the neurodevelopment and immunity of both foetus and the neonate.

Adolescence: At this stage of development, adequate nutrition, rich in both micronutrients and macronutrients, is required to ensure normal growth, hormonal regulation, and functions of the nervous system, including mental health.

Adulthood: Adequate nutrition is required to reduce the risks of hypertension, insulin resistance, and dyslipidemia among others.

Elderly: Adequate amounts of nutrients, such as B complex vitamins, antioxidants, calcium, vitamin D, among others, are essential to reduce incidence of osteoporosis, cognitive decline, frailty and sarcopenia.

Widely available global scientific health data and reliable practical considerations indicate that adequate nutrition is one of the most powerful and accessible preventive measures against poor health. However, adequate nutrition is not panacea. It cannot prevent all diseases. For example, infectious or congenital disorders, genetic predispositions, environmental exposures, poor lifestyle habits may require medical intervention. Adequate nutrition is effective when in synergy with other health promoting measures and conditions.

Despite the positive and widely acceptable impact of adequate nutrition, it remains elusive to many, especially in the resource limited countries. According to the WHO, food insecurity is a major obstacle because globally many people lack access to sufficient amounts of quality food. In addition, dietary choices are influenced by tradition, taste preferences, cultural and behavioral factors. Lack of nutrition counselling in communities is a major issue that needs attention.

Several researchers have proposed various methods to elevate adequate nutrition as a preventive tool. Some of the methods include:

- Increasing the nutrition-related components in the academic programs in medical training.
- Ensuring the empowerment of the people by educating them, through advocacy and campaign, about the importance of adequate nutrition and the benefits of consuming adequate diets. Include the people in decision-making regarding personalized dietary plans to enhance adherence.
- Reform existing policies by increasing subsidies and reducing costs for healthy foods, restricting labeling regulations, and introducing free lunches in schools, especially in the primary and elementary schools.

In conclusion:

Is adequate nutrition the best form of preventive medicine? It is not a panacea, but it is a powerful, evidence-based, biologically significant and universally accessible form of preventive medicine. It transcends age, geography, and socioeconomic status. Currently, the world is grappling with chronic disease epidemics, climate change, and healthcare inequities. Adequate nutrition is directly related to safer pregnancy and childbirth, improved infant, child and maternal health, to functional and robust immune system, and to lower risk of non-communicable diseases. People consuming adequate nutrition are more productive and can create opportunities to gradually break the cycles of poverty and hunger. Thus, adequate nutrition may be one of our most potent prescriptions for survival.

The emerging research in nutrigenomics demonstrates that dietary components interact and modulate gene expression, influencing disease susceptibility paving the way for personalized nutrition in the future, thus making preventive nutrition more accessible.

REFERENCE:

1. Elevina Perez Sira, Frederick Schroeder and Mily Monascal. Instituto de Ciencia y Tecnología de Alimentos, Facultad de Ciencias, Universidad Central de Venezuela, Venezuela. Food, Nutrition and Preventive Medicine. Premed Biology Department, Ball State University, USA. Education and Leadership Department, St. Mary University, Minneapolis, USA https://lupinepublishers.com/food-and-nutri-journal/pdf/SJFN.MS.ID.000112.pdf https://www.sciencedirect.com/topics/social-sciences/preventive-medicine
2. Afshin, A., Sur, P. J., Fay, K. A. Health effects of dietary risks in 195 countries, 1990–2017: A systematic analysis for the Global Burden of Disease Study 2017. The Lancet, 2019. 393(10184), 1958–1972. https://doi.org/10.1016/S0140-6736(19)30041-8
3. Calder, P. C. Nutrition, immunity and COVID-19. BMJ Nutrition, Prevention & Health, 4(1), 74 – 92. 2021. https://doi.org/10.1136/bmjnph-2020-000085
4. Chen, J., Gemming, L., Hanning, R., & Allman-Farinelli, M. Smartphone apps for dietary self-monitoring: A systematic review. JMIR mHealth and uHealth, 8(2), 2020. e16465. https://doi.org/10.2196/16465
5. Estruch, R., Ros, E., Salas-Salvadó, J. Primary prevention of cardiovascular disease with a Mediterranean diet supplemented with extra-virgin olive oil or nuts. New England Journal of Medicine, 2018. 378 (25), e34. https://doi.org/10.1056/NEJMoa1800389
6. Mozaffarian, D., Angell, S. Y., Lang, T., & Rivera, J. A. Role of government policy in nutrition—barriers to and opportunities for healthier eating. BMJ, 2018. 361, k2426. https://doi.org/10.1136/bmj.k2426
7. Ordovas, J. M., & Ferguson, L. R. Nutrigenomics and nutrigenetics. Annual Review of Nutrition, 2018. 38, 247–272. https://doi.org/10.1146/annurev-nutr-071715-050815
8. Popkin, B. M., Corvalan, C., & Grummer-Strawn, L. M. Dynamics of the double burden of malnutrition and the changing nutrition reality. The Lancet, 2020. 395 (10217), 65 – 74. https://doi.org/10.1016/S0140-6736(19)32497-3
9. World Health Organization. Global health estimates: Leading causes of death and disability. WHO, 2020.

HISTOMORPHOMETRIC STUDIES OF GARCINIA KOLA, MUSA PARADISIACA AND KHAYA IVORENSIS VS SURE MALE CAPSULE FORMULA DRUG (TRIUMVIRATE COMBINATION OF G. KOLA, M. PARADISIACA AND K. IVORENSIS) ON TESTICULAR FUNCTION IN ADULT MALE WISTAR RATS

YESSOUFOU IDRISSOU[1], OYEWOPO A. OYETUNJI*[2], OLUKAYODE O. ODUBELA[3], FLORENCE O OPOOLA4, GODWIN O. ADUNMO[5], CHIDUBEM F. EMEREONYE[6], COMFORT A. OLADELE[6], KEHINDE S. OLANIYI[6]

1. Yusram Herbal, Flower Street, Capital Park, Pretoria, Gautang, South Africa
2. *Anatomy, Department of Anatomy, University of Ilorin, Ilorin, Nigeria
3. Department of Anatomy, College of Health Sciences, Olabisi Onabanjo University, Sagamu Nigeria
4. Department of Anatomy, College of Medicine, Lagos State University, Ikeja, Nigeria
5. Department of Medical Lab Science, College of Health Science, University of Ilorin, Ilorin, Nigeria.
6. Department of Physiology, College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti, Nigeria.

***Corresponding author:** Oyewopo.oa@unilorin.edu.ng

Submitted: June 2025; Accepted: November 2025

ABSTRACT

This study evaluated the histomorphometric effects of *Garcinia kola*, *Musa paradisiaca*, *Khaya ivorensis*, and the Sure Male Capsule Formula—a commercial sexual stimulant comprising a combination of these three botanicals—on testicular function in adult male Wistar rats. Thirty rats were divided into five groups (n=6): Group A (control), Group B (500 mg/kg *M. paradisiaca*), Group C (200 mg/kg *G. kola*), Group D (200 mg/kg *K. ivorensis*), and Group E (400 mg/kg Sure Male Capsule Formula). Treatments were administered orally for 28 days. Post-treatment assessments included weight changes, semen parameters, histological and histopathological evaluations of testicular tissue using Johnsen's scoring system. Results revealed a significant increase in body weight in groups D and E ($P<0.05$). All treatment groups demonstrated varying degrees of testicular degeneration and reduced semen quality, including significant reductions in sperm count, motility, morphology, and viability. Histologically, all extracts showed degenerative changes in the seminiferous tubules, with the Sure Male Capsule Formula group showing pronounced architectural distortion and degeneration of interstitial and Leydig cells. However, Johnsen's scores were significantly lower in groups B and C, indicating severe spermatogenic disruption, while scores in groups D and E were comparable to the control, suggesting relatively preserved spermatogenesis. While individual plant extracts exhibited anti-spermatogenic and spermatotoxic effects, the Sure Male Capsule Formula showed some spermatogenic activity based on Johnsen's score alone. Nonetheless, its adverse histological profile cautions against its efficacy as a safe sexual stimulant. These findings underscore the need for further toxicological evaluation before clinical application.

Keywords: Sure, Male Capsule Formula, testicular function, *Garcinia kola*, *Musa paradisiaca*, *Khaya ivorensis*, spermatogenesis, Wistar rats.

INTRODUCTION

Methanolic extract of *M. paradisiaca* flower showed significant reduction in blood glucose levels in streptozotocin induced diabetic rats and reduced oxidative stress, the superoxide dismutase, lipids, peroxide and catalase in nephropathy and in testicular groups. Therefore, the extract has reno-protective and testicular-protective effect [1]. Mallick *et al.* [2], showed that hexane fraction of root of *M. paradisiaca* has the potential to correct diabetes induced testicular germ cell apoptosis. Ethanolic extract of Garcinia kola on the histology of the testes of male adult Wistar indicated that it had some adverse effects on the testis of male Wistar rats which was dose dependent [3]. G. kola has however been reported to cause deterioration of the reproductive system by Braide *et al.* [4]. Nottidge *et al.* [5] also reported prolonged administration of bitter kola extract to have destructive effects on the testes of dogs.

Akingboye *et al.* [6] reported that the consumption of the ethanolic extract of Garcinia kola at high doses may cause histopathological lesions in the testicular cells. Udoh [7] reported that the long-term ingestion of Garcinia kola seed would result in the arrest of spermatogenesis and sperm degeneration. Reports have shown that components of Garcinia kola seeds caused a toxic effect on the sertoli cells, have a negative impact on the leydig cells thereby interfering with testosterone production leading to reduction in spermatogenesis [8].

Khaya ivorensis (African mahogany or Lagos mahogany) is used as a bitter tonic, remedy for fever, vermifuge and for treating venereal diseases [9]. It is used as an antimalaria, as well as having anthelmintic effects [10]. Extracts of the stembark of *K. ivorensis* are used as anticonvulsants and treatment of hemorrhoids, heat rash, boils and arthritis [11].

Male Capsule Formula is a drug used for stimulating male reproductive hormone in man. It Contains 30 capsules, average weight of one capsule 0.33g. Constituents include K*haya ivorensis, Garcinia kola and Musa paradisiaca. Note:* The drug is still on trial basis to ascertain its potency on sexual function by examining the various constituents.

Therefore, the aim was to evaluate the histomorphometric studies of *garcinia kola, musa paradisiaca, khaya ivorensis versus* Male Capsule Formula drug (triumvirate combination of *garcinia kola, musa paradisiaca, khaya ivorensis*) on testicular function in adult male Wistar rats.

METHODOLOGY

Ethical Review

These investigations were carried out in conformity with the rules and guidelines of the

Animal Ethics Committee of the University of Ilorin, Ilorin. Kwara State, Nigeria.

Animal Grouping

Thirty (30) adults male Wistar rats, average weight of 200g were obtained from a commercial Animal Holding Facility in Ogbomoso, Oyo state, Nigeria. The rats were maintained in the Animal House of the Basic Medical Science, College of Health Sciences, University of Ilorin. Kwara State. The rats were fed with food and water ad libitum. They were acclimatized for one week before commencement of the experiment and weekly weighing of the animals was done using an Electronic Weighing Balance in the Department of Anatomy, College of Health Sciences, University of Ilorin Kwara State. The rats were grouped into four, with each group consisting of four rats each. The grouping was as follows:

Group A – Control group; received 1ml of distilled water, Group B- *Musa paradisiaca* 500 mg/kg body weight daily, Group C- Garcinia Kola 200mg/kg body weight daily, group D- *Khaya Ivorensis* 200mg/kg body weight daily and group E- Sure Male Capsule Formula 400mg/kg body weight daily.

Preparation and Administration of the Extract

Musa paradisiaca

Green plantain fruits were obtained from Ipata Market in Ilorin, Nigeria. The fruits were cut longitudinally into chips of about 5 mm thickness and air-dried for 4 days after which they were grinded and made into flour [12]. The *musa paradisiaca* flour was dissolved in 2 ml of double distilled water, for easy administration while a dosage of 500 mg/kg/day was given to the group using an oral cannula [12]. The duration of administration was 28 days.

Garcinia Kola

Garcinia Kola seeds were bought from the Ipata Market in Ilorin, Nigeria. The outer coats were removed and the seeds cut into pieces and air-dried. The dried seeds were ground to fine powder. The dried powdered 21g *Garcinia kola* was dissolved in 421ml of distilled water and shaken vigorously; it was allowed to stand on the bench shaking at intervals. The solution was then refrigerated for twenty-four (24) hours and then sieved using laboratory sieve. This was then allowed to stand for at least one (1) hour to allow the heavier particles to settle down after which, it was decanted and filtered using Whatman filter paper while the filtrate was collected into a bottle [9]. Extract was administered orally using an oral cannula. Dosage of 200mg/kg body weight daily was administered for 28 days, and the animals were sacrificed 24 hours after [9, 13].

Khaya Ivorensis

K. Ivorensis stem bark was purchased from Ipata Market, Ilorin, Nigeria. The *Khaya Ivorensis*

extract was prepared at the Chemistry Laboratory, Department of Chemistry, University of Ilorin.

Sure, Male Capsule Formula

The Sure Male Capsule used was purchased from a dealer (fig 1). Sure Male Capsule formula contains 66 grams which was dissolved in 1 Liter of water to obtain a concentration of 0.06g/L. Rats in this group received 400mg/kg body weight of Male capsule formula daily.

The solution was prepared by using the formula.

$$\text{Volume of X (ml)} = \frac{\text{Weight of rat (kg) x dosage (mg/ml)}}{\text{Concentration of X (mg/ml)}}$$

Assuming X represented male capsule formula

Sacrifice of Animals

Twenty-four hours after the 28th day of administration the rats were sacrificed by cervical dislocation. Thereafter, the testis was identified and excised while the caudal epididymis was immediately removed for semen analysis. The testis removed were fixed in 10% formalin to prevent putrefaction and autolysis for histological analysis.

Semen Analysis

The epididymis was placed in normal and used for evaluation of sperm quality (i.e. sperm count, sperm motility and sperm morphology). The concentration of spermatozoa was determined using the improved Neubauer Chamber Haemocytometer (Deep 1/10 mm, LABART, Germany)

Histological Analysis

The testes of all the rats were fixed in 10% formalin, dehydrated stepwise in graded ethanol, cleared in xylene and then embedded in paraffin wax. A section of 5µm thick paraffin section of each testicular tissue was stained with hematoxylin and eosin (H &E), followed by examination under a Olympus binocular research microscope (Olympus, New Jersey, USA) which was connected to a 5.0MP Amscope Camera (Amscope Inc, USA.) at ×100 magnification.

Histopathological Evaluation of the Testis

In addition to qualitative descriptions, the testicular histology was analyzed quantitatively using modified

Johnsen's Score [14]. The Johnsen's Score grades seminiferous tubules on a scale of 1-10 so that the best histological appearance with evidence of full spermatogenesis is rated 10 while the worst histological appearance with absent seminiferous tubule is given a score of One.

The score allocated to each seminiferous tubule is based on the descriptions summarized below. The modified Johnsen's scores were applied to a minimum of 25 randomly chosen seminiferous tubules from each animal. The mean score for

each animal was obtained by multiplying the numbers of tubules awarded a score with the score and then, the total number of tubules graded was divided by the sum of all the multiplications. The group mean was obtained by dividing the sum of means by number of animals per group.

Modified Johnsen's Scores as described by Holstein *et al*. [14].

10	*Represents intact spermatogenesis with many mature spermatids and zones of spermiation.*
9	*Describes modestly reduced spermatogenesis: reduced number of mature spermatids, a few zones of spermiation.*
8	*Implies a distinct reduction in spermatogenesis: few mature spermatids, no spermiation.*
7	*Is awarded when there is considerably reduced spermatogenesis: no mature spermatids, only immature spermatids, no spermiation.*
6	*Means severely reduced spermatogenesis: only few immature spermatids, reduced height of germinal epithelium.*
5	*Indicates arrest of spermatogenesis at the stage of primary spermatocytes: many spermatocytes border the lumen of the seminiferous tubules.*
4	*Implies arrest of spermatogenesis at the stage of primary spermatocytes: a few primary spermatocytes are present.*
3	*Depicts arrest at the stage of spermatogonia: A-type spermatogonia multiply but do not develop to maturing cells of spermatogenesis*
2	*Means there are no germ cells but only Sertoli cells are present.*
1	*Awarded when neither germ cells nor Sertoli cells are found. The seminiferous tubule is also replaced by connective tissue ground substance.*

Statistical Analysis

Data from both groups were statistically analyzed using Student's t-test followed by subsequent analysis by GraphPad Prism software (version 5.02) with statistical significance set at $P<0.05$* and $P<0.001$**. The mean, the standard error of mean and standard deviation of the data were calculated.

RESULTS

Physical Observations

The treated groups exhibited some behavioral changes such as aggressiveness in group B, agitation, restlessness, and disorientation in group C while group E exhibited calmness and tranquility compared to the control group. Reduced food intake was observed in all the treatment groups (B, C, D and E) compared to the control group.

Weight Changes

Significant increase ($P<0.05$) in weight changes was observed in groups D and E when compared to the control (table 1).

Semen Analysis

Significant decrease ($P<0.05$) in sperm count was observed in groups B, C and E when compared to the control group. Significant decrease ($P<0.05$) in

Sperm Motility was observed in groups D and E compared to the control group (Fig. 1). Sperm morphology experienced a significant decrease (P<0.05) in groups C and D compared to the control group (Fig. 2). Significant decrease (P<0.05) in Life and Death ratio was observed in groups D and E compared with the control group (Table 2).

Table 1: Showing the weight changes of the treatment groups compared to the control group.

Groups	Initial weight (g)	Final weight (g)	Weight difference (g)
A	175.0 ± 1.3	185.0 ± 3.0	10.0 ± 0.72
B	165.0 ± 3.22	180.0 ± 3.56	15.0 ± 0.34
C	170.0 ± 3.22	180.0 ± 3.23	10.0 ± 0.55
D	175.0 ± 3.54	195.0 ± 3.23	20.0 ± 0.42*
E	160.0 ± 3.34	185.0 ± 3.78	25.0 ± 0.34*

* = P <0.05 = Significant difference.

Table 2: Semen analyses of rats in the treatment groups compared to the control group.

Groups	Sperm Count (%)	Sperm motility (%)	Sperm Morphology (%)	L/D ratio (%)
A	70.70±0.94	86.66±2.75	86.87±2.14	86.96±2.64
B	55.76±3.63*	76.3±1.36	79.40±1.40	76.40±3.64
C	58.4±1.22*	83.9±2.84	76.53±2.63*	72.79±2.33
D	61.9±3.63	75.14±1.43*	69.14±1.34*	71.14±1.44*
E	60.4±3.51*	65.44±2.25*	81.40±2.31	69.78±2.21*

*= (P<0.05) - significant difference.

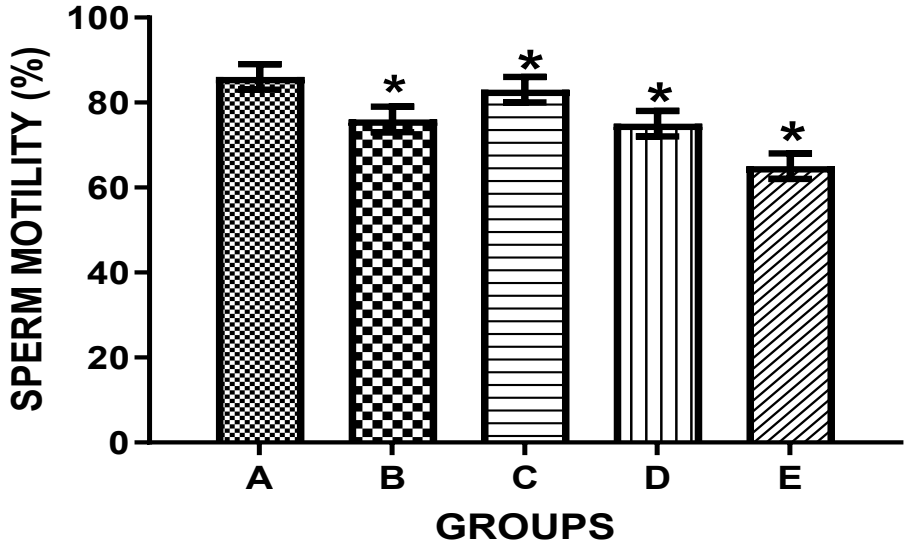


Fig 1: Sperm motility of rats in the treatment groups compared with the control group. *(P<0.05)-significant difference.

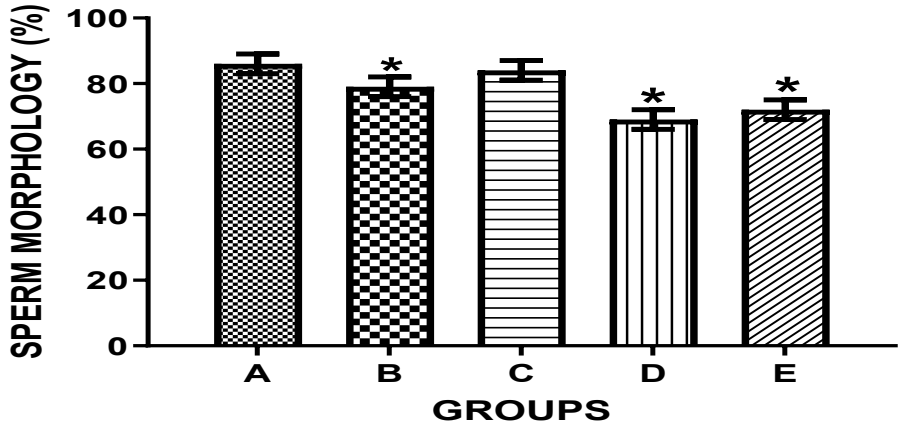


Fig 2: Sperm morphology of rats in the treatment groups compared with the control group. *(P<0.05)-significant difference.

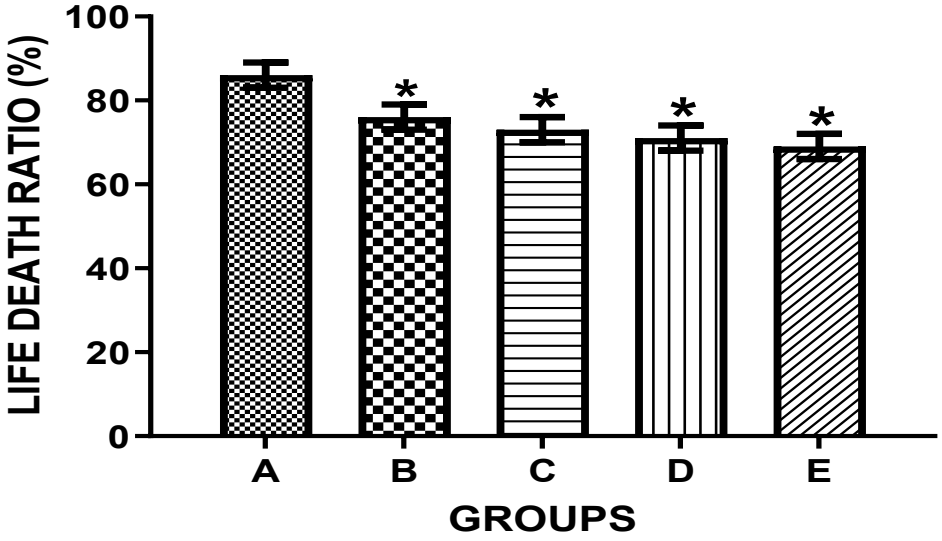


Fig 3: showing the Life Death Ratio of rats in the treatment groups compared with the control group.
*(P<0.05)-significant difference.

Histological Observation

The control group (A) showed well-defined seminiferous tubules, interstitial cells (IC) and a well-spaced lumen (L). The *Musa paradisca* (B) group showed slight degeneration of seminiferous tubules and epithelium, degenerated and widened lumen with variable cells around the tubules. The *Garcina kola* (C) group showed a defective seminiferous epithelium, degenerated basement membrane, detachment of sertoli cells and reduced numbers of spermatogenic cells. The *Khaya ivorensis* (D) group showed a severe degenerated seminiferous epithelia highlighting severe alterations in their architecture.

The Male Capsule Formula (E) group showed poor seminiferous tubule profile, distorted arrangement of the spermatogenic cells, increased interstitial space, detachment of sertoli cells, severe degeneration of the interstitial cells and Leydig cells (Fig. 4).

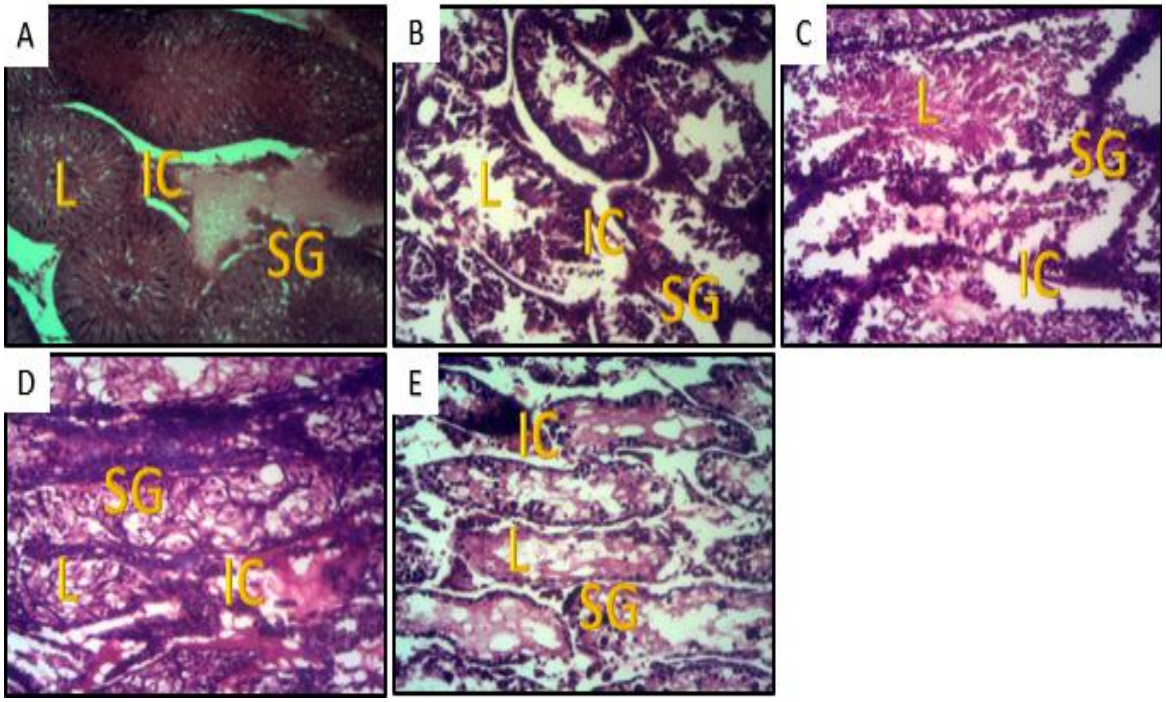


Fig. 4: (A-E) Photomicrograph representative of the testis of adult male Wistar rats treated with normal saline, control (A). Musa paradisca (B), Garcina kola (C), Khaya ivorensis (D), and Male Capsule Formula (E). L-lumem, IC- Interstitial Cells, SG-spermatogonia. (H and E x100).

Histopathological Observation

Histopathological observations using Johnsen's Score showed that were significant reduction (***P<0.001) in groups B and C when compared to the control group while no significant difference was observed in groups D and E which showed normal spermatogenesis in some seminiferous tubules (Table 3). This depicted a slight reduction in the number of germ cells present in all treatment groups (B, C, D and E).

Table 3: Histomorphometric observation across treatment and control groups using Johnsen's Score

A	B	C	D	E
8.4	5.5	6.4	8.7	8.8
8	4.6	6.1	8.8	8.9
7.56	5.3	5	8.7	8.8

DISCUSSION

Significant increase (P<0.05) in weight differences was observed in groups D and E when compared to the control (table 1). This negates studies by Akingboye *et al.,* [9] who observed significant decrease (P<0.001) in the final body weights and body weight differences after investigating the effects of ethanolic extract of *Garcinia kola* on the testes of adult male Wistar rats. Results from groups B and C were in consonance with Abu e*t al.* [15], Ralebona *et al*. [16] and Sumita *et al.* [17] who observed no significant difference in body weight. However, dose dependent reduction was observed in the weight of the testis, epididymis, prostate and seminal vesicle after investigating the effects of ethanolic extract of *Garcinia kola* and *Musa paradisiaca* on serum sex hormones and testicular function respectively.

The significant decrease in semen parameters observed in all treatment groups were in consonance with studies by Akpantah *et al*., [15], Adesanya *et al.* [18] and Abu *et al.,* [19] who observed marked reduction in sperm concentration, motility, viability and morphology after administration of ethanolic extract of G. *kola* on the testis to investigate its antispermatogenic effects (table 2) (Fig 1) (Fig 2). Significant decrease in Life and Death ratio was observed in groups D and E compared with the control group was consistent with the findings of Iwuji and Herbert [20] who demonstrated significant decrease in abnormal sperms in all the treated groups (Fig 3) [20]. They further stated that a decrease in the abnormalities of the head and tail in all the treated groups when compared with the controls was noted. It contradicted Ralebona *et al.* [16] who reported that G. kola treatment enhances testosterone secretion and increases testicular weights which accounts for the enhanced sexual activity, improve libido and intensifying orgasm and ejaculation and it's likely due to the presence of antioxidants such as kolaviron in the G kola extract. Therefore, these reductions in sperm parameters may suggest an impairment of the hypothalamo-pituitary-gonadal axis [21]. It is also probable that the reduction in sperm concentration and motility may be due to a reduction in gonadotropin levels which in turn suppressed testosterone secretion thus suggesting an antispermatogenic property.

The degenerated seminiferous tubules observed in all treatment groups agreed with Braide *et al*. [4] who reported that G. kola caused a severe deterioration of the reproductive system. Nottidge *et al.* [5] also reported prolonged administration of bitter kola extract to have destructive effects on the testes of dogs. Udoh [7] reported that the long-term ingestion of *Garcinia kola* seed would result

in the arrest of spermatogenesis and sperm degeneration. Reports have also shown that components of *Garcinia kola* seeds caused a toxic effect on the sertoli cells, having a negative impact on the leydig cells thereby interfering with testosterone production leading to reduction in spermatogenesis [7, 8].

According to Johnsen's Score reported in this study, groups B and C had a significantly low score compared to the control. This is an indicator that administration of extracts in both groups was not very healthy for male reproductive health. This was corroborated by the poorly arranged histoarchitecture of the testicular profile in this group. This result agrees with Udoh [7] who reported that the prolonged use of *Musa paradisica* could lead to disruption of the spermatogenic process. While Johnsen's Score in groups D and E was at par with the control thereby suggesting both extracts might be good for reproduction, but this negates reports from the histological observation in both groups.

The investigation was conducted in adherence to the National Institute of Health Guide for the Care and Use of Laboratory Animals, and the study protocol was approved by the University Ethical Review Committee (UERC). Consent to participate is not applicable.

Competing interests

The authors declare no competing interest.

REFERENCES

1. Vinaykumar T, Sumanth MH, Suman L, Vijayan V, Srinivasarao D, Sharmila A. Renoprotective and testicular protective effect of Musa paradisiaca flower extract in streptozotocin-induced diabetic rats. J Innov Trends Pharm Sci. 2010;1(2):106.
2. Mallick C, Bera TK, Ali KM, Chatterjee K, Ghosh D. Diabetes-induced testicular disorders vis-a-vis germ cell apoptosis in albino rat: remedial effect of hexane fraction of root of Musa paradisiaca and leaf of Coccinia indica. Journal Health Sci. 2010; 56 (6): 641-54.
3. Ajibade AJ, Oyewopo AO, Fakunle PB, Ashamu EA. Effect of crude ethanolic extract of Garcinia kola on the testes of adult male Wistar rats. WAJAR. 2011;2(1):32.
4. Braide VB, Agube CA, Essien GE, Udoh FV. Effect of Garcinia kola seed extract on the level of gonadal hormones and pituitary gonadotropins in rat serum. Nig J Physiol Sci. 2003;18(1-3):59-64.
5. Nottidge HO, Omobowale TO, Taiwo VO, Omotoso MA. Histopathological studies on the effects of the ethanolic extract of the fruits of Garcinia kola on selected organs of the dog. Int J Morphol. 2008;26(4):1067-72.
6. Akingboye AJ, Ezejindu DN, Udemezue OO, Chukwujekwu IE, Anyabolu AE, Ofoego UC. The effects of ethanolic extract of Garcinia kola on the testes of male adult Wistar rats. J Nat Sci Res. 2015;5(3):184-90.
7. Udoh FV. Effect of extract of Garcinia kola seeds on reproductive organs of male rats. M.Sc. Thesis, University of Calabar, Calabar, Nigeria; 1998. p. 132.
8. Naiho AO. Effects of Garcinia kola on the reproductive organ weight and histology of

the testis and anterior pituitary gland of Wistar rats. J Exp Clin Anat. 2004;3:20-3.
9. Iwu MM. Handbook of African medicinal plants. Boca Raton: CRC Press; 1993.
10. Olayinka AO, Onabanjo OO, Lot TY. Effects of Khaya senegalensis purinergic transmission in the rat bladder. Pharm Biol. 1994;32(4):346-51.
11. Mshana NR, Abbiw DK, Addae-Mensah I, Adjanouhoun EA, Ahyi MRA, Ekpere JA. Traditional medicine and pharmacopoeia: contribution to the revision of ethnobotanical and floristic studies in Ghana. 2nd ed. Publication of the Scientific, Technical and Research Commission of the Organization of African Unity (OAU/STRC); 2000. p. 137-419, 732-733.
12. Anosa GN, Okoro OJ. Anticoccidial activity of the methanolic extract of Musa paradisiaca root in chickens. Trop Anim Health Prod. 2011 Jan; 43: 245-8.
13. Alaka OO, Olaniyi MO, Mshelbwala FM, Oni OO, Adebowale OO, Akintunde OG. Pathological, biochemical and hormonal changes associated with prolonged administration of methanol extract of Garcinia kola seed in adult male Wistar rats. Niger Vet J. 2022; 43 (4): 36-49.
14. Holstein A, Schulze W, Davidoff M. Understanding spermatogenesis is a prerequisite for treatment. Reprod Biol Endocrinol. 2003; 1: 107.
15. Abu AH, Amuta PO, Buba E, Inusa TR. Evaluation of antispermatogenic effects of Garcinia kola seed extract in albino rats. Asian Pac J Reprod. 2013;15-18.
16. Ralebona N, Sewani-Rusike CR, Nkeh-Chungag BN. Effects of ethanolic extract of Garcinia kola on sexual behaviour and sperm parameters in male Wistar rats. Afr J Pharm Pharmacol. 2012;6(14):1077-82.
17. Sumita B, Sanjib B, Pandey JN, Moulisha B. Acute and sub-chronic toxicity study of Musa paradisiaca leaf extracts in mice. J Adv Pharm Edu Res. 2013;3(2):90-3.
18. Akpantah AO, Oremosu AA, Ajala MO, Noronha CC, Okalawon AO. The effects of crude extract of Garcinia kola seed on the histology and hormonal milieu of male Sprague Dawley rats' reproductive organs. Nig J Health Biomed Sci. 2003; 2: 40-6.
19. Adesanya OA, Oluyemi KA, Olusori DA, Omotuyi IO, Okwuonu CU, Ukwenya OV. Micromorphometric and stereological effects of ethanolic extracts of Garcinia cambogia seeds on the testes and epididymides of adult Wistar rats. Int J Alt Med. 2007;5(1):1-9.
20. Iwuji TC, Herbert U. Semen characteristics and libido of rabbit bucks fed diets containing Garcinia kola seed meal. Int J Bioflux Soc. 2012;2(1):10-14.
21. Lohiya NK, Goyal RB, Jayaprakash D, Ansari AS, Sharma S. Antifertility effects of aqueous extract of Carica papaya seeds in male rats. Planta Med. 1994; 60:400-4.

NEUROPATHIC PAIN MANAGEMENT IN INDIA: A CROSS-SECTIONAL STUDY OF PRESCRIPTION PRACTICES IN INDIA

ARIF A. FARUQUI

Clinical Pharmacologist Department of Pharmacology, Rizvi Mahal CHS Opp. K. B. Bhabha Hospital Bandra West, Mumbai, Maharashtra, India

Correspondence: drfaruqui@gmail.com

Submitted: April 2025. Accepted: October 2025

ABSTRACT

Neuropathic pain presents significant clinical challenges due to its complex pathophysiology and varied patient response to treatment. While multiple pharmacologic options are available, healthcare provider's preferences and prescribing behaviors can influence treatment outcomes and patient adherence. This study aimed to evaluate the preferences of healthcare providers in India and their current prescription trends in the management of neuropathic pain. A cross-sectional survey was conducted among 755 practicing healthcare professionals across India, comprising 376 orthopedic specialists and 379 physicians. A structured questionnaire was used to assess preferred drug molecule from tricyclic antidepressant class, factors affecting drug selection (e.g., perceived efficacy, patient tolerability, cost), preferred combination and prescribing trends of Nortriptyline, Pregabalin and Mecobalamin (NPM) combination. Descriptive statistics were used to analyze the responses. Nortriptyline was the most preferred tricyclic antidepressant for neuropathic pain (40.98%), followed by amitriptyline (33.26%) and protriptyline (15.66%). Efficacy was the primary determinant in drug selection (45.92%), followed by tolerability (32.29%), compliance (18.88%), and cost (7.61%). The NPM combination was frequently prescribed, especially for diabetic neuropathy, though concerns about cost and safety influence its adoption. Healthcare providers (HCPs) generally prescribe this combination for 1-3 months, but adherence was a challenge, with only 13.30% of patients showing adherence rate of over 75%. The 40-60 years age group was the primary demographic for NPM prescriptions. Cost and size of the tablet/capsule were pivotal factors in brand selection. Nortriptyline was the preferred tricyclic antidepressant for neuropathic pain among Indian healthcare providers, with efficacy, tolerability, and compliance being key factors in drug selection. The NPM combination was commonly prescribed, especially in diabetic neuropathy, despite concerns regarding cost and safety. Patient adherence to this regimen was relatively low, highlighting the need for strategies to improve long-term compliance.

Keywords: Neuropathic pain, nortriptyline, pregabalin, mecobalamin, combination.

INTRODUCTION

Neuropathic pain (NP) is a complex, chronic pain condition resulting from damage or disease affecting the somatosensory nervous system. It is estimated to affect up to 10% of the global population and is frequently associated with

conditions such as diabetic neuropathy, post-herpetic neuralgia, spinal cord injuries, and chemotherapy-induced peripheral neuropathy [1,2]. Unlike nociceptive pain, neuropathic pain is often resistant to conventional analgesics, necessitating the use of adjuvant therapies such as antidepressants, anticonvulsants, and combination regimens [3].

Tricyclic antidepressants (TCAs), particularly amitriptyline and nortriptyline, have long been used as first-line agents in NP management due to their dual action on noradrenergic and serotonergic pathways, which modulate pain perception [4]. Pregabalin, a gabapentinoid, is also widely prescribed owing to its efficacy in reducing neuronal excitability by modulating calcium channels [5]. Furthermore, mecobalamin (methylcobalamin), a neurologically active form of vitamin B12, is frequently included in treatment combinations due to its role in nerve regeneration and myelin repair [6].

Despite the range of therapeutic options available, the clinical management of neuropathic pain is often hindered by heterogeneous treatment responses, adverse effect profiles, patient non-adherence, and cost-related barriers. In this context, the prescribing behavior and preferences of healthcare providers (HCPs) play a critical role in optimizing treatment outcomes. Factors such as perceived efficacy, side-effect profile, tolerability, and drug accessibility can significantly influence drug selection and treatment duration [7,8]. India presents a unique landscape in neuropathic pain management due to its diverse patient population, varying socioeconomic conditions, and evolving healthcare infrastructure. However, limited data exist on how HCPs in India approach the pharmacologic management of NP, particularly with respect to preferred agents within the TCA class, the use of fixed-dose combinations like Nortriptyline, Pregabalin, and Mecobalamin (NPM), and the practical considerations that guide these decisions.

This study aimed to address this gap by conducting a cross-sectional survey among physicians and orthopedic specialists across India to identify current prescription trends, drug preferences, and influencing factors in the management of neuropathic pain. Understanding these patterns is essential for informing future guidelines, improving adherence strategies, and tailoring interventions that align with real-world clinical practices.

METHODOLOGY

Study Design and Setting:

This was a cross-sectional, questionnaire-based survey conducted among practicing orthopedics and consulting physicians involved in the diagnosis and management of neuropathic pain across India. Participation was voluntary, and confidentiality of responses was assured. The goal was to gather real-world insights on current prescription trends, preferred combination and prescribing trends of Nortriptyline, Pregabalin

and Mecobalamin (NPM) combination in neuropathic pain management. The study was approved by the Altezza Institutional Ethics Committee (IEC) having Protocol Number: 2024/MPL/O/01.

Survey Participants:

The participants included orthopedics and consulting physicians who routinely manage patients with neuropathic pain. A total of 755 participants responded to the questionnaire. Inclusion criteria (1) Licensed healthcare providers. (2) Actively involved in prescribing neuropathic pain medications. (3) Willing to provide informed consent for participation. All data were collected prospectively, and all participants responded to a uniform 10-item questionnaire. Participants voluntarily completed the questionnaire after being informed about the research objectives, providing full consent to participate. The study involved no patient contact or clinical intervention.

Application of Questionnaire:

A structured 10-item questionnaire was developed to assess orthopedics and consulting physician's preferences and perceptions regarding neuropathic pain management, particularly focusing on tricyclic antidepressants and nortriptyline, pregabalin and mecobalamin combination (Table 1). The survey questionnaire was proposed by the author of the study and is not a validated tool in neuropathic pain management.

Table 1: Questionnaire for Healthcare Professionals

No.	Questions	Options
1	Preferred Molecule Among Tricyclic Antidepressants	Nortriptyline
		Amitriptyline
		Protriptyline
		Other
2	Reason for Molecule Preference	Efficacy
		Safety Profile
		Compliance
		Cost
		Any Other
3	Common Indication for Nortriptyline, Pregabalin, and Mecobalamin (NPM)Combination	Diabetic Neuropathy
		Chronic Pain
		Spinal Cord Injury
		Post-Herpetic Neuralgia
		Low Back Pain
4	Frequency of Prescribing NPM Combination	25-50% of cases

		10-25%of cases
		<10% of cases
		>50% of cases
5	Major Concerns for Not Prescribing NPM Combination	Cost
		Safety
		Compliance
		Efficacy
6	Duration of NPM Prescription	1 month
		2-3 months
		15 days
		>3 months
7	Patient Adherence to NPM Prescription	25-50% adherence
		50-75% adherence
		<25% adherence
		>75% adherence
8	Most Preferred Molecule/Combination	Nortriptyline + Pregabalin + Mecobalamin
		Nortriptyline + Pregabalin
		Plain Nortriptyline
		Any Other
9	Common Age Group f or NPM Prescription	40-60 years
		60-75 years
		25-40 years
		>75 years
10	Important Brand Features f or NPM Combination	Cost
		Size of Tablet/Capsule
		Availability
		Onset of Action/Technology
		Frequency of Reminders

Data Collection:

The questionnaire was administered through interviews over a 3-months period. Respondents were encouraged to answer all items honestly based on their current clinical practices and beliefs. No identifying personal or institutional information was collected to maintain confidentiality.

Statistical Analysis:

Data from the completed questionnaires were entered into Microsoft Excel and analyzed using descriptive statistics. Categorical variables were expressed in percentages and frequencies. No inferential statistics were applied, as the

objective was to assess trends and opinions rather than test hypotheses.

RESULTS

A cross-sectional survey of 755 healthcare professionals, including 376 orthopedic specialists and 379 physicians, revealed key insights into prescribing trends and preferences in the management of neuropathic pain in India. Among tricyclic antidepressants, nortriptyline was the most preferred molecule (40.98%), followed by amitriptyline (33.26%) and protriptyline (15.66%). Efficacy was the leading factor influencing molecule selection (45.92%), followed by safety (32.29%), compliance (18.88%), and cost (7.61%). The fixed-dose combination of nortriptyline, pregabalin, and mecobalamin was most commonly prescribed for diabetic neuropathy (43.88%), with other indications including chronic pain (29.72%), spinal cord injury (19.00%), low back pain (15.00%), and post-herpetic neuralgia (11.37%). In terms of prescribing frequency, nearly half of the respondents (47.96%) reported using the NPM combination in 25–50% of NP cases, while 22.74% used it in 10–25%, and 12.12% in more than 50% of cases. The most cited barriers to prescribing NPM included high cost (34.12%), safety concerns (27.46%), compliance issues (24.67%), and perceived efficacy (22.42%). Most providers prescribed the NPM combination for a duration of 1 month (38.30%) or 2–3 months (33.04%). However, patient adherence to therapy remained suboptimal, with only 13.30% of clinicians reporting adherence rates above 75%, and the majority indicating 25–50% adherence (39.05%). Regarding formulation preferences, nortriptyline + pregabalin + mecobalamin was favored by 39.27% of respondents, while 36.26% preferred nortriptyline + pregabalin, and 17.16% prescribed nortriptyline alone. The most common age group receiving NPM prescriptions was 40-60 years (53.32%), followed by 60–75 years (23.28%) and 25–40 years (19.20%). Key brand selection criteria included cost (36.00%), tablet/capsule size (26.18%), availability (24.57%), onset of action or formulation technology (19.00%), and reminder frequency (11.48%). These findings underscore both the clinical rationale behind prescribing behaviors and the practical challenges faced in optimizing neuropathic pain management.

Both healthcare professional groups showed a clear preference for nortriptyline and amitriptyline with orthopedics favoring nortriptyline more prominently. Efficacy drives molecule preference across both groups, with orthopedics placing slightly more emphasis on safety, while consulting physicians consider compliance and cost marginally more. Both groups identified diabetic neuropathy as the leading indication, with orthopedics slightly more focused on chronic pain and low back pain. Both groups prescribed NPM combination at moderate frequencies, while consulting physicians used it slightly more in 25-50% of cases. Cost remained the primary barrier for

both groups; consulting physicians expressed slightly more concern about safety and compliance. Orthopedics favored shorter durations (1 month), while consulting physicians were more likely to prescribe for 2-3 months. Both groups faced significant adherence challenges, with adherence rates between 25-75% being most common. Both healthcare professional groups favored the NPM combination and predominantly prescribed it for patients aged 40-60 years. Cost was the top priority for both groups, with minor differences in preferences for other features.

DISCUSSION

The findings from this cross-sectional survey of 755 healthcare professionals in India provide valuable insights into the prescribing patterns, preferences and challenges associated with the management of neuropathic pain, particularly with the use of the nortriptyline, pregabalin, and mecobalamin fixed-dose combination. The preference for nortriptyline (40.98%) over amitriptyline (33.26%) and protriptyline (15.66%) among tricyclic antidepressants aligns with global trends favouring nortriptyline due to its favourable side-effect profile compared to amitriptyline. For instance, a systematic review by Finnerup et al. [3] highlighted nortriptyline's efficacy in NP management with fewer anticholinergic side effects, making it a preferred choice for conditions like diabetic neuropathy and post-herpetic neuralgia. This preference was particularly pronounced among orthopaedic specialists (46.27%) in our study, likely due to their focus on safety, which corroborates findings from a European study by Gray et al. [9], where safety concerns significantly influenced TCA prescribing decisions.

Efficacy emerged as the primary driver for molecule selection (45.92%), consistent with guideline recommendations from the International Association for the Study of Pain (IASP), which emphasize evidence-based efficacy for TCAs and anticonvulsants like pregabalin in NP management [10]. The frequent use of the NPM combination for diabetic neuropathy (43.88%) reflects its established role in addressing this prevalent condition in India, where diabetes affects over 77 million individuals [11]. The combination of nortriptyline, pregabalin, and mecobalamin likely offers synergistic effects, with pregabalin targeting neuropathic pain through calcium channel modulation and mecobalamin supporting nerve repair, as supported by clinical studies [12]. However, the use of this combination for other indications, such as chronic pain (29.72%) and low back pain (15.00%), suggests a broader application, potentially driven by clinical experience rather than robust evidence, as these indications lack strong guideline support as per latest evidence-based recommendations from Attal et al., [13].

The moderate prescribing frequency of the NPM combination (47.96% in 25–50% of NP cases) indicates cautious adoption, possibly due to barriers such as high cost (34.12%), safety

concerns (27.46%), and suboptimal patient adherence (39.05% reporting 25–50% adherence). Cost as a primary barrier is particularly relevant in the Indian context, where out-of-pocket healthcare expenditure remains high as reflected by a study from Garg & Karan et al., [14]. This finding echoes studies from low- and middle-income countries, where cost significantly limits access to NP medications [15]. The slightly higher emphasis on safety among orthopedic specialists and on compliance among consulting physicians may reflect their differing patient populations and clinical priorities. Orthopedists, often managing musculoskeletal-related NP, may prioritize safety to minimize adverse effects in patients with comorbidities, while physicians, dealing with diverse NP etiologies, may focus on compliance to ensure therapeutic continuity.

Patient adherence remains a critical challenge, with only 13.30% of clinicians reporting adherence rates above 75%. This aligns with literature highlighting poor adherence to NP medications due to side effects, complex regimen, and socioeconomic factors [16]. The preference for shorter treatment durations (1 month by 38.30%) among orthopedics compared to 2-3 months among physicians suggests differing approaches to balancing efficacy and adherence, potentially influenced by patient follow-up patterns. The predominance of NPM prescriptions in the 40-60 age group (53.32%) reflects the high burden of diabetic neuropathy and chronic pain in this demographic, consistent with epidemiological data from India [17].

Formulation preferences, with 39.27% favoring the NPM combination and 36.26% opting for nortriptyline + pregabalin, suggest a tailored approach to NP management. The inclusion of mecobalamin in the NPM combination may be driven by its perceived neuroprotective benefits, though evidence remains mixed [6]. Brand selection criteria, led by cost (36.00%) and tablet/capsule size (26.18%), underscore practical considerations in prescribing decisions, particularly in resource-constrained settings. These findings align with studies indicating that formulation characteristics, such as ease of administration, influence prescribing behavior in chronic conditions [18].

The differences between orthopedic specialists and consulting physicians, while subtle, highlight the need for tailored educational interventions to optimize NP management. Orthopedics preference for shorter durations and greater focus on safety may reflect their surgical orientation and shorter patient interaction times, whereas physicians' emphasis on compliance and longer durations may stem from managing chronic conditions in outpatient settings. Future research should explore these differences through qualitative studies to better understand decision-making processes.

Limitations of the study:

This study has limitations, including its cross-sectional design, which limits causal inferences,

and its reliance on self-reported data, which may introduce bias. Additionally, the survey did not capture patient perspectives, which are critical for understanding adherence challenges. Future studies should incorporate longitudinal designs and patient-reported outcomes to validate these findings and assess the real-world effectiveness of the NPM combination. Moreover, cost-effectiveness analyses could guide policy interventions to improve access to NP medications in India.

CONCLUSION

In conclusion, the survey highlights the prominence of nortriptyline-based regimens, particularly the NPM combination, in managing neuropathic pain in India, driven by efficacy and tempered by cost and adherence challenges. These insights underscore the need for strategies to enhance affordability, improve patient adherence, and align prescribing practices with evidence-based guidelines to optimize NP management.

Acknowledgement:

Author acknowledges the contribution of Mr. Rafique Sheikh, M. Pharm (Pharmacology) for his contribution to doing literature search & helping with the preparation of manuscript.

REFERENCES

1. Colloca L, Ludman T, Bouhassira D, Baron R, Dickenson AH, Yarnitsky D. Neuropathic pain. *Nat Rev Dis Primers*. 2017; 3:17002.
2. van Hecke O, Austin SK, Khan RA, Smith BH, Torrance N. Neuropathic pain in the general population: a systematic review of epidemiological studies. *Pain*. 2014;155(4):654-662.
3. Finnerup NB, Attal N, Haroutounian S, McNicol E, Baron R, Dworkin RH, et al. Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. *Lancet Neurol*. 2015; 14 (2): 162-73.
4. Moore RA, Derry S, Aldington D, Cole P, Wiffen PJ. Amitriptyline for neuropathic pain in adults. *Cochrane Database Syst Rev*. 2015;2015(7):CD008242.
5. Ziegler D, Fonseca V. From guideline to patient: a review of recent recommendations for pharmacotherapy of painful diabetic neuropathy. *J Diabetes Complications*. 2015;29(1):146-56.
6. Sawangjit R, Thongphui S, Chaichompu W, Phumart P. Efficacy and Safety of Mecobalamin on Peripheral Neuropathy: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *J Altern Complement Med*. 2020;26(12):1117-1129.
7. Hange N, Poudel S, Ozair S, Paul T, Nambakkam M, Shrestha R, et al. Managing Chronic Neuropathic Pain: Recent Advances and New Challenges. *Neurol Res Int*. 2022; 2022:8336561.
8. Saxena AK, Jain P, Dureja GP, Venkitachalam A, Goswami S, Usmani H, et al. Pharmacological management of neuropathic pain in India: A consensus statement from Indian experts. *Indian J Pain*. 2018;32:132-44.
9. Gray L, Beddard M, Jones S, Begum A, Azhar NB, Deslandes P, et al. Trends in tricyclic antidepressant prescribing and

poisoning in England and Wales 2016-2020. *Br J Clin Pharmacol*. 2025.

10. Dworkin RH, O'Connor AB, Audette J, Baron R, Gourlay GK, Haanpää ML. Recommendations for the pharmacological management of neuropathic pain: an overview and literature update. *Mayo Clin Proc*. 2010;85(3 Suppl): S3-14.
11. Muralidharan S. Diabetes and current Indian scenario: a narrative review. *J Diabetol*. 2024;15(1):12-17.
12. Raju N, Villavan S, Ravi S. Clinical effectiveness and treatment satisfaction between two triple-therapy regimens in treating neuropathic pain: a real-world data. *ibrain*. 2023; 1-14.
13. Attal N. Pharmacological treatments of neuropathic pain: The latest recommendations. *Rev Neurol (Paris)*. 2019;175(1-2):46-50.
14. Garg CC, Karan AK. Reducing out-of-pocket expenditures to reduce poverty: a disaggregated analysis at rural-urban and state level in India. *Health Policy Plan*. 2009;24(2):116-28.
15. Morriss WW, Roques CJ. Pain management in low- and middle-income countries. *BJA Educ*. 2018;18(9):265-270.
16. Cardoso MG, Weinstock, JG, Sardá Júnior, JJ. Adhesion to neuropathic pain treatment. *Revista Dor*. 2016;17((suppl 1)):107-109.
17. Karthiksaravanan K, Meriton AS. A study on prevalence of diabetic peripheral neuropathy in diabetic patients attending a rural health and training centre. *J Family Med Prim Care*. 2024;13(2):726-729.
18. Chethan Kumar JK, Jason Fernandes Jokem J, Chandralekha N and Udaykumar P. Neuropathic Pain Management: Prescription Strategies in a Tertiary Care Setting. National Board of Examinations - *J Med Sci*. 2025;3(1):7-21.

OSTEOGENESIS IMPERFECTA INVOLVING A MOTHER AND HER TWO DAUGHTERS WITH A REVIEW OF LITERATURE.

Short title: **Osteogenesis imperfecta in siblings**

DANIEL A. NDIDIAMAKA ONYIRIUKA AND VASHTI EDOSUYI

Endocrinology and Metabolism Unit, Department of Child Health, University of Benin Teaching Hospital, Benin City, Nigeria.

Corresponding author: alpndiony@yahoo.com; didiruka@gmail.com

Submitted: August 2025. Accepted: October 2025

ABSTRACT

In children, fractures following minimal trauma is the most prevalent sign and a hallmark of osteogenesis imperfecta (OI). Long bones, particularly in the lower limbs, are frequent fracture sites in children with OI. The diagnosis is based mainly on history, physical findings and radiographic features. In this report familial clustering, the clinical and epidemiological characteristics of two female siblings (including their mother) with OI are described while the newer classification, the differential diagnosis and the aims of therapy are also emphasized.

Key words: Collagen disorders, dentinogenesis imperfecta, familial clustering, osteogenesis imperfecta.

INTRODUCTION

Osteogenesis Imperfecta (OI) (also called Brittle Bone Disease) is a heterogeneous inherited metabolic bone disorder characterized by short stature, long bone deformity, and susceptibility to fracture from mild or inconsequential trauma due to reduced strength and increased brittleness of the bones [1]. It is a generalized disorder of connective tissue and the most common genetic cause of primary osteoporosis in childhood [2]. It has been proposed that OI be defined as a syndrome of congenital brittle bones secondary to mutation in the genes codifying for pro-collagen genes (COL1A1 and COL1A2) [3]. Majority of cases (approximately 90%) are due to genetic mutations involving the two genes, collagen type 1, alpha 1 (COL1A1) on chromosome 17q21 and collagen type 1, alpha 2 (COL1A2) on chromosome 7q22.1 [4,5]. Most of the mutations that cause OI type 1 occur in COL1A1 gene. Many of the other rare forms of OI are due to defects in protein involved in cross-linking, hydroxylation, and mineralization of type I collagen [2]. Type 1 collagen is a major

protein constituent of bone, dentin, skin, sclera, blood vessels and heart valves and plays a major role in the pathogenesis of OI. Essentially, there are two molecular mechanisms resulting in OI caused by collagen mutation [6]. The first mechanism is through chain exclusion in which the mutant chain is not incorporated into collagen triple helix, resulting in the milder OI type 1 because the abnormal microfibril is unable to incorporate into the triple helix and is thus degraded, leaving the remaining allele to produce less structurally intact collagen triple helix. The second mechanism is through chain non-exclusion in which abnormal collagen chain results in a defective helix (a qualitative defect). The resultant dominant negative effect manifests as more severe OI types II, III and IV. Worldwide, the incidence of OI is one in 20,000 live births and the prevalence is six to seven per 100,000 [7] and its frequency appear similar in all ethnic and racial groups. Some familial recurrences of OI are due to parental mosaicism for dominant collagen mutation [2]. Classical OI (Sillence types I-IV) are inherited as autosomal dominant disorder, so also is OI type V [2]. The diagnosis of OI is established by clinical criteria and confirmed by genotyping of COL1A1 and/or COL1A2 or other pertinent genes. However, failure to detect a genetic mutation does not necessarily rule out OI [8]. Diagnosing the various genes is challenging due to variability in clinical manifestations and the high degree of overlap in phenotypes. In literature, the variable clinical manifestation has been linked to factors such as epigenetics, modifiers and environmental influences [9].

Regarding the aetiopathogenesis of OI collagen, fibres are usually oriented in a preferential direction with hydroxyapatite crystals located in the ground substance within these fibres. The hydroxyapatite crystals provide mechanical rigidity and strength to the bone whereas the fibres provide resilience. Individuals with OI have either less or poorer (or both) quality type I collagen fibres than unaffected people, causing their bones to deform or fracture (or both) [10]. The Sillence classification of 1979, divides OI into four types (I-IV) based on clinical and radiographic findings [11]. However, this classification has two major drawbacks which are the overlapping nature among different types and the difficulty in using it for prognosis based on the type. Therefore, caution is advised in the use of the numeric classification of OI and the severity must always be assessed in each individual case. In 2014, this classification was revised with introduction of OI type V, characterized by interosseous ossification with propensity to develop hyperplastic callus [12]. However, this new classification system still retains the Sillence classification for defects associated with mutations in type 1 collagen genes. Genomic tests can be done with collagen analysis from fibroblast.

Among the oral manifestations of OI, dentinogenesis imperfecta (DGI) is the most prominent [13]. According to Shield's classification, there are three types; DGI type 1

(DGI-1), a syndromic form, always occurs in association with OI while DGI types II and III, non-syndromic forms, are not associated with OI [14,15]. The morbidity and mortality of OI involve the cardiopulmonary system. Recurrent pneumonias and declining pulmonary function occur in childhood and a chronic lung disease (cor pulmonale) may be seen in adults. Neurologic complications include basilar invagination, brainstem compression, hydrocephalus and syringohydromyelia. Apart from these complications, OI is a potentially incapacitating clinical condition, interfering negatively with quality of life (QoL).

The purpose of this report is to raise awareness among clinicians of this rare clinical entity, the familial clustering nature and psychosocial impact. Additionally, to review the literature to highlight its differential diagnosis as well as challenges in diagnosis and management.

Case presentation

Case 1: A 5-year-old girl, born at term, to non-consanguineous parents, presented to our hospital with a history of poor growth noticed from the age of 2 years and recurrent fracture of the arms. First episode of fracture of left arm was 3 years ago while the child was being massaged at home. Second episode was one year ago following a fall while attempting to walk. Mother noticed poor growth since the age of 2 years, being markedly smaller than other children younger than her. She can neither stand nor walk, making it impossible for her to enroll in school. She has no hearing difficulty.

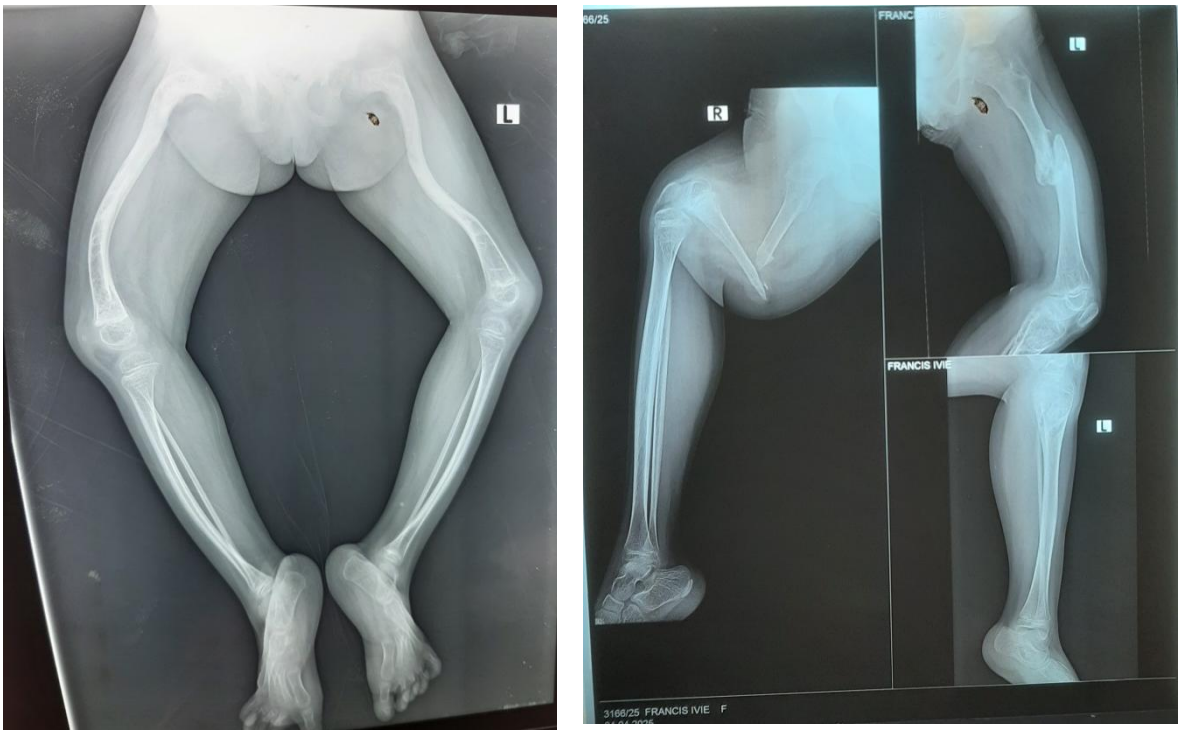
Physical examination revealed small-for-age stature, triangular facie and skull deformity. The auxological findings include length 75.0cm (< 3rd percentile, using OI specific growth chart), weight 9.5kg (< 3rd percentile), BMI 16.9kg/m^2 (75th percentile) occipitofrontal circumference 47.cm, Arm length 9.5cm, Forearm length 12.5cm, Chest circumference 43.5cm. Her vital signs were within normal limits. Additional findings include pectus carinatum, dentinogenesis imperfect, skeletal deformities of the long bones. Abdominal examination revealed hepatomegaly. Cutaneous signs of physical abuse were absent. The radiographic findings include thickening of the diploid space of the left parietal bone, defective modeling and shortening of both humeri, generalized osteopaenia with thinning of the ribs, defective modeling of the femur bilaterally with bowing of the femur and tibia bilaterally.

Case 2: A 9-year-old girl, born at term, to non-consanguineous parents, presented with fracture of the femur 6 months ago and inability to walk since then. This is the second episode of fracture in this patient. The fracture occurred following a fall having been pushed by another child. She did not fall from a height. The first episode of fracture was 6 years ago at the age of 3 years. The fracture occurred when she attempted to walk and fell. She has no hearing

impairment. On examination, her weight was 15kg (< 3rd percentile), length was 90cm (< 3rd percentile) and BMI 18.5kg/m^2 (75th percentile). She had deformity and shortening of right lower limb. Part of the broken femur was protruding posteriorly covered only by a scar. She also has dentinogenesis imperfecta. There are no bruises at the site of fracture. The patient has stopped schooling because of progressive deformity and fractures of the lower limbs.

The family/social history was remarkable. The mother of the two siblings also has a positive history of fracture of left femur following a fall while walking after the rain at the age of 10 years. The mother (a petty trader) has no formal education, and she does not know her age. The father has primary school education, and he is a carpenter. However, he abandoned the children and their mother because of their clinical condition. The patients' medical care is being sponsored by a kind-hearted man. On physical examination of the mother, it was noted that she has short stature (height 145cm) and weighed 40kg. She has skull deformity (like that of case 1) and dentine dysplasia. Having clinically made a diagnosis of OI, the patients commenced on vitamin D supplementation 400units daily because it is known that serum 25-hydroxy-vitamin D is often low, because of immobility and ultimately, insufficient exposure to sunlight. The orthopaedic surgeon and physiotherapist were invited to form a multidisciplinary healthcare team.

Figure 1: Case 1 shows deformities of femur and tibia



Case 1 shows long bone deformities **Case 2 shows fracture of femur**

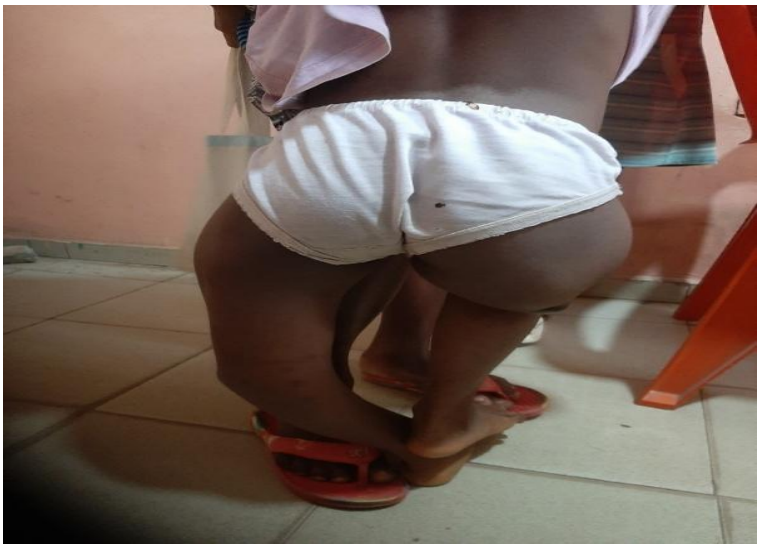


Figure 2 shows deformities of low limbs in case 2.

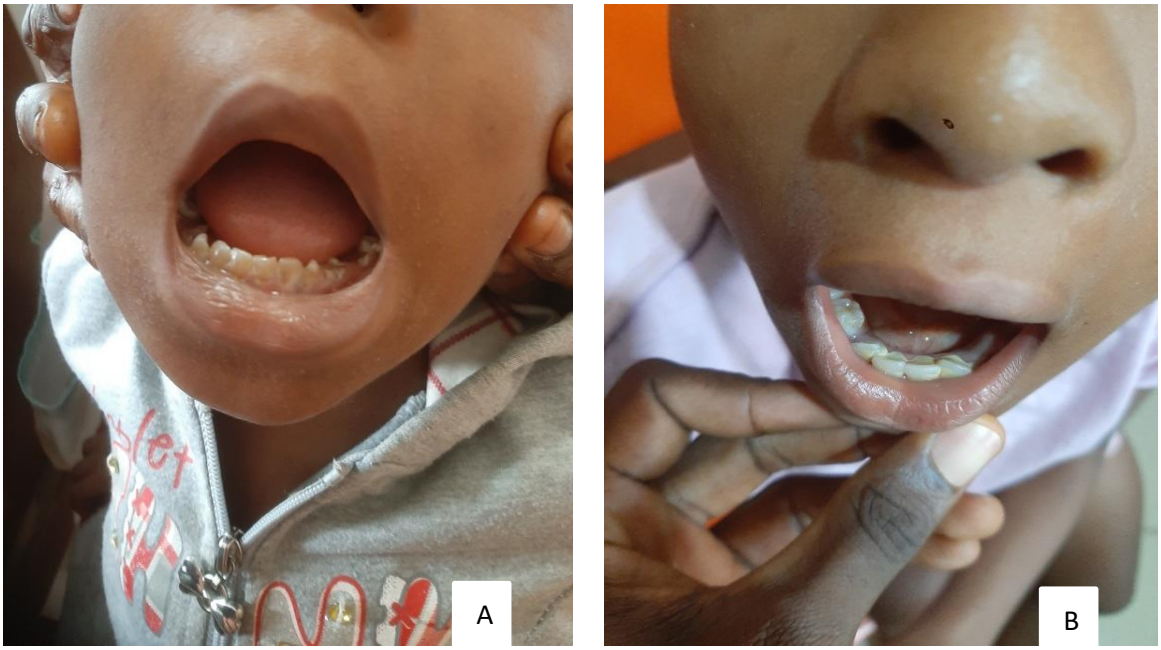


Figure 3a: shows dentine abnormalities: A: Case 1 **B: Case 2**

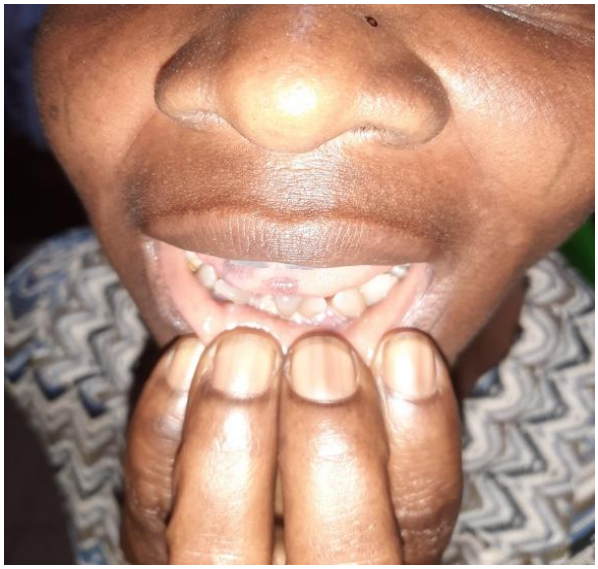


Figure 3b: shows dentine abnormalities: C: Mother

Table 1: Summary of investigation results for cases 1 and 2.

Laboratory parameters	Case 1	Case 2	Reference intervals
Serum calcium	7.6mg/dl	7.9mg/dl	8.0 - 10.5
Serum organic phosphate	3.5mg/dl	4.0mg/dl	4.0 - 7.5mg/dl
Alkaline phosphatase	105iu/L	102iu/L	≤ 270
Serum urea	10mg/dl	9.6mg/dl	10.0 - 50.0
Serum sodium	139mmol/L	140mmol/L	135 – 145
Serum potassium	4.9mmol/L	4.5mmol/L	3.5 - 5.5
Serum bicarbonate	19.0mmol/L	20.0mmol/L	20 – 30
Serum chloride	100.0mmol/L	102.0mmol/L	99 – 107
Serum creatinine	0.4mg/dl	0.3mg/dl	0.6 - 1.4
Aspartate aminotransfarse	56iu/L	48iu/L	≤ 40
Alanine aminotransferase	28iu/L	26iu/L	≤ 40
Total bilirubin	0.7mg/dl	0.5mg/dl	0.2 - 1.2
Conjugated bilirubin	0.3mg/dl	0.3mg/dl	0.2 - 0.6
Non-conjugated bilirubin	0.4mg/dl	0.2mg/dl	0.1 - 0.2
Total protein	6.7g/dl	7.0g/dl	6.0 - 8.2
Serum albumin	3.8.g/dl	4.0g/dl	3.3 - 5.1
Serum globulin	2.8g/dl	3.0g/dl	2.0 - 3.2
Packed cell volume	37.0%	40.0%	33 – 50

DISCUSSION/LITERATURE REVIEW

In this report, the diagnosis of OI was based on history of repeated fractures secondary to mild trauma, positive history of pathologic fractures in a first degree relative (mother), physical examination findings (severe short stature, dentinogenesis imperfect) and radiographic findings of lower limb fractures, long bone deformities as well as generalized osteopaenia. In summary, the known triad of OI (namely, fracture from minor trauma, long bone curvature deformity and growth deficiency) were present in the two siblings. The hallmark of OI in children is fractured due to minimal trauma and this history was positive in the two siblings including their mother, further reinforcing the diagnosis. Lack of adequate laboratory facility prevented performing both the collagen biopsy test and the DNA test which are thought to detect nearly 90% of all type 1 collagen mutations [16].

It is noteworthy that the first case sustained fracture of the upper limb rather than the lower limb which typically is involved in fractures. In addition, the two siblings presented here exhibited clinical features in keeping with Sillence OI type III, which is characterized by severe short stature, progressive skeletal deformities, recurrent pathologic fractures with minimal trauma, scoliosis, triangular-shaped face, basilar skull deformities, dentinogenesis imperfecta as well as thinning of the ribs. Applying the flowchart for clinical diagnosis of OI suggested by the Indonesian Paediatric Association Clinical Practice Guideline on OI, it further supported the diagnosis of OI type III in the two siblings presented here [17]. The possibility that the index cases are OI type III is further reinforced by the report of a study in South Africa regarding its relative prevalence in Blacks. In that report, a study involving children from South Africa and Zimbabwe showed that OI type III is relatively common in an indigenous Black population [18]. The results of a study in South Africa showed that among indigenous black population of South Africa, the minimum population frequency of OI type III was 6 per 100,000 [19].

In addition to these characteristics, the mother also has a positive history of pathological fracture at the age of 10 years, indicating familial clustering. Physical examination of the mother showed dentine dysplasia, skull deformity and triangular-shaped face similar to that of the observed in Case 1, suggesting that the mother also has OI, probably of a lesser severity than that of her offspring. In this regard, most likely OI type 1 (the most common subtype of OI) or type IV (intermediate severity). Thus, illustrating the heterogeneous nature of OI. This finding is supported by a recent report by Cruz-Centeno et al [20] in Puerto Rico in which a mother and her two children (a male and a female) had OI. In the present report, the mother and her two daughters are affected. It has been documented that majority (approximately 90%) of cases of

type 1 collagen mutation involve autosomal dominant mode of inheritance [4,5]. There is no history of consanguinity in parents. The father did not have similar history or physical features. In a comprehensive review article, the authors stated that the differential diagnosis of OI is largely determined by the age of presentation and clinical severity [7].

In that review, the stated common differential diagnosis of OI includes osteopaenia of prematurity, hypophosphataemia, idiopathic juvenile osteoporosis and non-accidental injury. The two siblings (index cases) were full term deliveries, making osteopaenia of prematurity unlikely.

In hypophosphataemia, serum phosphate is low while alkaline phosphatase is elevated. In the two siblings, these biochemical parameters were within normal limits, and the typical radiographic features (flaying, splaying, cupping) of rickets were absent, making hypophosphataemia less likely. Idiopathic juvenile osteoporosis (IJO) presents in previously healthy prepubertal children (usually 2 to 3 years before puberty) and resolves spontaneously over 2 to 5 years [21].

This disorder (IJO) is not inherited, therefore familial clustering is not expected. Non-accidental injury may be distinguished by metaphyseal, rib and skull fractures. Such pattern of fracture was absent in the two siblings presented and there was no history suggestive of non-accidental injury. Bruises which are typical of non-accidental injury were absent on examination of the skin of these two siblings. Also, multiple injuries and fractures at various stages of healing were absent. The risk of non-accidental injury is inversely related to age, with majority of the victims being below two years of age [22].

In contrast, the index cases were aged 5 and 9 years, respectively. These findings further make non-accidental injury less likely. Further details are shown in Table 2 [23] and it outlines the key features of the common differential diagnosis.

Table 2: Common differential diagnosis of osteogenesis imperfect

Clinical condition	Clinical features
Non-accidental injury	May be distinguished by metaphyseal, rib and skull fractures and presence of bruises.
Rickets	Distinguished by typical radiographic features of fraying, splaying cupping at metaphysis. Widening of the wrist and ankle.

Infantile Hypophosphatasia	Presents with a low alkaline phosphatase levels and spurs extending from sides of the knee and elbow joints. Presence of excessive excretion of phosphorylethanolamine in urine.
Campomelic dwarfism (compomelic dysplasia)	Congenital bowing and angulation of long bone may be mistaken for OI but fractures are not common. They have peculiar facial anomalies (flat face, long philtrum, micrognathia). Survival beyond the newborn period is rare.
Achondroplasia	Rhizomelia and enlarged head, radiographs sufficient to differentiate
Idiophatic juvenile Osteoporosis	The disorder is characterized by its prepubertal onset (2 to 3 years before puberty) and resolve spontaneously after puberty.

Adapted from Phonela et al [23]

In the present report, dentine abnormalities were observed in both the mother and her two daughters. The results of a study by Sillence et al [11] indicated that dentinogenesis imperfecta (DGI) was present in 50% of cases of OI, making it a relatively common finding in patients with OI. Similarly, in a study in Vietnam involving 68 children aged 3 to 17 years with OI, 47.1% of them had DGI [24]. The two siblings (females) had dentinogenesis imperfecta; a clinical feature which has been reported to be more common in girls than boys [25]. The results of a study by Yamaguti et al [26], showed that dentinogenesis imperfecta is a common finding in OI and that its frequency is higher in patients with COL1A2 than in those with COL1A1 mutation. In this context, is it possible that the index cases have COL1A2 instead of COL1A1 mutation? Sillence et al [11] observed that DGI is more common in OI type III than other subtypes. Similarly, Andersson et al [27] reported a higher prevalence (86%) of DGI among children and adolescents with OI type III. In this context, the results of the two studies increased the possibility that the two siblings reported here may have OI type III. This view is further supported by literature in which it was stated that abnormal dentition has been observed in 80% of children less than 10 years of age with OI type III [20]. The two siblings presented here are below 10 years of age. Additionally, it is documented in literature that OI type III is an autosomal-dominant trait due to point or frame-shift mutations in COL1A1(Gly154Arg, Gly844Ser) and COL1A2 (Gly526Cys) [20]. Appropriate dental care can lead to improved control of oral disease, function and esthetics in DGI.

There exists a wide variation in clinical characteristics of different types of OI, among people with the same type of OI, and within members of the same family with a particular type of OI. In the present report, the mother did not have any repeat episode of fracture since

the first at the age of 10 years, illustrating the variability in severity. However, she has dentine dysplasia, triangular-shaped face, skull deformity, and short stature resembling those present in younger child (Case1). In sum, these features suggest that the mother has undiagnosed OI but does not have deformity of long bones and reoccurrence of fractures noted in her two daughters. This variation in clinical findings is in keeping with what has been documented in literature which stated that the mutation will usually be identical in a given family but its expression (i.e., the degree of severity and the number of fractures) may differ among members [16]. This characteristic points to the importance of molecular diagnosis which is useful for counseling on prognosis, recurrence, and heritability as well as for variable response to drugs [11]. In the index cases, there was no molecular diagnosis because of lack of laboratory facility for that purpose in the health facility where we practice. Some of the clinical features present in the index cases and their scientific bases are worthy of note. The triangular facial shape is due to overdevelopment of the head and underdevelopment of the face bones [28]. The brittle teeth (dentinogenesis imperfecta) is one of the most important oral findings in type III OI and inherited in an autosomal dominant fashion. Dentinogenesis imperfecta (DGI) is characterized by presence of opalescent yellow brown-coloured brittle teeth and can affect both primary and secondary dentition as illustrated in the index cases. The enamel may be normal in thickness but gets dislodged easily because of smooth dentinoenamel junction, exposing the softer dentin [29]. Genetic analyses have found two subgroups of DGI namely, DGI type 1, syndromic form which is associated with OI and DGI type II, non-syndromic form that is not associated with OI [30,31]. The progressive deformities of long bones result from frequent fractures of long bones, tension of muscles on soft bones, and the disruption of growth plates. Children with OI type III have severe short stature and as adults are shorter than 102cm [28]. The increased curvature of the long bones leads to increase in maximum stresses within the bone shaft. Such increase in stresses attributed to bone deformities in OI might contribute to occurrence of bone fractures. The altered structure of the growth plates gives a popcorn-like appearance to the metaphyses and epiphyses. In Case 1, pectus carinatum was present but absent in case 2, illustrating variability in manifestation of OI in a given family. The total serum calcium was slightly reduced in both siblings presented here. Vitamin D and calcium are vital components of skeleton, and their deficiency can worsen the osteopaenia caused by OI. Therefore, vitamin D supplementation at maintenance dose of 400units daily was prescribed for the patients. In individuals with OI, outdoor activity decreases due to limited mobility and the resultant insufficient exposure to sunlight may contribute to vitamin D deficiency.

Regarding gender, the two siblings presented here are females. Although in literature, it is stated that there is no significant gender difference in incidence of OI, many cases reported from Nigeria, Akiola et al [32] in Lagos and Ogundare et al [33] in Ado Ekiti were all females. So also, was the case reported by Bastos et al [34] in Angola. In consonance, the results of a study in Iran involving 23 children showed that 69.1% were females and the rest were males [25]. In a nation-wide data analysis in Taiwan involving 319 patients with validated OI, 52% were females [35]. Even in the context of subtypes of OI, Aglan et al [36] in Egypt observed that among 24 children with OI type III, 66.7% were females while the rest were males. All pointing towards slight female preponderance.

Consistent with the results of the study by Lund et al [37], the two siblings and their mother had short stature. In that study, it was emphasized that the frequency and severity of growth retardation were more in patients with OI types III and IV. Typically, by the age of 2 to 3 years the growth retardation becomes obvious with stature below the third centile [38]. The two siblings being reported here have stature less than third percentile. However, the patients' mother was unable to state the age at which growth failure was noticed in her children as she was more concerned with the fractures. The poor linear growth and short stature in patients with OI has been linked to skeletal abnormalities such as lower limb bowing and extremity fractures, scoliosis, vertebral compression and growth plate disintegration ("pop-corn epiphyses" typical of severe OI) [3]. In that report, it was stated that short stature is the hallmark of moderate and severe OI. In addition, defective osteoblastic/bone matrix feedback on growth hormone-IGF 1 axis has been suggested as a mechanism for the short stature observed in OI types III and IV [37,39].

Apart from the classification suggested in 1979 by Sillence [11], newer classification of osteogenesis imperfecta by the International Nomenclature Group for Constitutional Disorders ICHG of the Skeleton (INCDS) (and preferred by Orthopaedic Surgeons) proposed that OI syndromes be classified into five different groups based on phenotype alone. Consequently, five clinical types of OI are generally recognizable with routine diagnostic methods, namely history, clinical examination and radiographs.

The division of OI according to the INCDS classification uses Roman nomenclature for identification and uses Arabic nomenclature to indicate phenotypes [12] and this is outlined in Table 3 (INCDS classification).

Table 3: The International Nomenclature Group for Constitutional Disorders ICHG of the Skeleton (INCDS).

New OI classification/OI type	Phenotype
1/I	Mild non-deforming
2/II	Severe, seen as perinatal and lethal forms
3/III, VI, VIII, IX, X, Bruck syndrome Type 1	Moderately severe, progressive deforming
4/IV, IV, VII, XI, XII, XIII	Moderate
5/V, osteoporosis-pseudoglioma syndrome, Idiopathic juvenile osteoporosis, Bruck syndrome Type 1 and Type 2	Presence of moderate calcification of interosseous membranes.

OI = Osteogenesis imperfect. Adapted from [12]

Healthcare issues in OI type III include the need to prevent fracture cycles, the need to develop strategies to cope with short stature, fatigue and the family's need for emotional support. It is equally important to address difficulties with social integration, participation in leisure activities and maintaining stamina [28]. In hospitals, non-invasive blood pressure cuff should be positioned carefully, but in some patients intra-arterial catheter is needed.

An important but often overlooked aspect of management of children with OI is the psychological care of the patients and their families. In Case 1, she has never walked at the age of 5 years and has never been enrolled in school. Although Case 2 was able to walk initially but lost the capacity by the age of 9 years and as a consequence, dropped out of school. Thus, illustrating the psychosocial problems of OI on the sufferers as well as its adverse effects on quality of life. The parents were deeply sad because of the physical disability of their children. This feeling progressed to helplessness and depression. A similar finding was reported in South Africa by Stephen et al [40]. With the financial support provided by a kind-hearted individual who is now sponsoring the healthcare of the two siblings the mother expressed some happiness. Childhood OI imposes a huge financial and psychological burden on the sufferers and their families as illustrated in the present report. The parents of the two siblings not only must perform their daily duties, roles and obligations but also meet the specific needs arising from their children's clinical conditions. This causes serious disruption of the family dynamics [41]. In the index cases the mother is the prime caregiver having been abandoned by their father. The potential effect on the mother is physical and psychological exhaustion due to accumulation of functions at home [42]. As a result, this mother can develop health problems and can be considered a "hidden patient" even if she did not

have OI. One of the strategies to assess the impact of the child's disease on the caregiver is by performing an assessment of the quality of life (QoL). Such assessment of QoL is based on the following aspects: subjectivity, multidimensionality and the presence of positive (i.e., mobility) and negative (i.e., pain, dimensions) [43].

Treatment of OI poses a serious challenge because the available treatment modalities do not target the underlying collagen defect. Modes of therapy vary depending on severity of the disease, degree of impairment, and age of the individual. The goals of pharmacological therapy include reducing associated pain and risk of reoccurrence of fractures as well as accelerating growth, improving bone metabolic indicators, bone histomorphometry, bone mineral density, improving mobility and independence [44-46]. Other therapeutic modalities in OI include physical therapy, rehabilitation and orthopaedic surgery. All these modalities of treatment are aimed at improving the quality of life of both the child and their primary caregivers. It must be emphasized that the chances of achieving these goals depend heavily on a well-coordinated multidisciplinary approach.

CONCLUSION

The heterogeneous nature of clinical manifestations of OI presents serious challenges in diagnosis and management, particularly in resource-poor countries, highlighting the need for increased awareness among clinicians. Some clinical features such history of fracture of long bones with minimal trauma, positive family history and physical findings such as dentinogenesis imperfecta and blue sclera, where present, may aid diagnosis. In general, diagnosis depends heavily on clinical features and radiographic findings. In low- and middle-income countries conventional radiography is a key to identifying the hallmark features OI such as generalized osteopaenia or osteoporosis, long bone deformities and multiple fractures.

Multidisciplinary approach is indispensible for successful management. In this context, the team of specialists should include paediatricians, geneticists, endocrinologists, orthopaedic surgeons, physiotherapists, and nutritionists to handle the various needs of the patient.

Funding: We did not receive any funding.

No conflict of interest.

REFERENCES

1. Unger S, Ferriera CR, Mortier GR, Ali H, Bertola DR, Calder A. Nosology of genetic skeletal disorders: 2023 revision. Am. J Med Genet A. 2023;191:1164-1209. doi:10.1002/amjg.a.63123.
2. Marini JC. Osteogenesis Imperfecta. In: Nelson Textbook of Paediatrics Volume 2, 20th edition, Kliegman RM, Stanton BF, St Geme JW III, Schor NF, editors, Philadelphia, Elsevier, 2016:3380-3384.
3. Plotkin H. Syndromes with congenital brittle bone. BMC Pediatrics 2004;4:16 doi:10.1186/1471-2431-4-16.

4. Roth KS, Ward RJ, Chan JCM, Sarafoglou K. Disorders of calcium, phosphate and bone metabolism. In: Paediatric Endocrinology and Inborn Errors of Metabolism. Sarafoglou K, editor, New York, McGraw Hill Companies, Inc 2009:619-664.
5. Rauch F, Glorieux FH. Ostegenesis imperfecta. Lancet 2004; 363:1377-1385.
6. Basel D, Steiner RD. Osteogenesis imperfecta: Recent findings shed new light on this once well-understood condition. Genetics in Medicine. 2009; Vol 11 number 6, page 376.
7. Van Dijk FS, Cobben JM, Kariminejad A, Maugeri A, Nikkels PGJ. Osteogenesis imperfect: a review with clinical examples. Mol Syndromol 2011; 2(1):1-20.doi 10.1159/000332226.
8. Root AW, Diamond FB. Disorders of mineral homeostasis in the newborn, infant, child and adolescent. In: Pediatric Endocrinology, 3rd edition, Sperling MA editor, Philadelphia, Saunders Elsevier, 2008:686-769.
9. Umair M, Ahmad F, Abbas S. Syndactyly genes and classification. JBC Genetics 2018; 1:10-18.
10. McCarthy EF. Genetic diseases of bones and joints. Semin Diagn Pathol 2011;28(1):26-36.
11. Sillence DO, Senn A, Danks DM. Genetic heterogeneity of osteogenesis imperfect. J Med Gen 1979; 10:101-116. doi 10.1136.jmg.16.2.101.
12. Van Dijk FS, Sillence DO. Osteogenesis imperfecta. Clinical diagnosis, nomenclature and severity assessment. Am J Med Genet 2014;164A(6):1470-1481.
13. Opsahl Vital S, Gaucher C, Bardet C, Rowe PS, George A, Linglart A, Chaussain C. Tooth dentin defects reflect genetic disorders affecting bone mineralization. Bone 2012; 50:989-997.
14. Shields E, Bixler D, El-Kafrawy A. A proposed classification of human dentine defects with description of a new entity. Arch Oral Biol 1973; 18:543-553.
15. Forlino A, Marini JC. Osteogenesis Imperfecta. Lancet 2016; 387:1557-1571. doi: 10.1016/S0140-6736(15)00728-X.
16. National Institutes of Health, Osteoporosis and Related Bone Diseases – National Resource Centre, USA. Guide to Osteogenesis Imperfecta for Paediatricians and Family Practice Physicians. Gloriex F. Editor, 2007.
17. Arimbawa LM, Himawan LW, Rini ABPEA, Marzuki NS, Batubara JR. Indonesian Paediatric Association Clinical Practice Guideline on Osteogenesis Imperfecta. 1st edition, Indonesian Paediatric Association Publishing Agency. 2017.
18. Beighton P, Versfield GA. On the paradoxically high prevalence of osteogenesis imperfecta type III in Black population in South Africa. Clin Genet 1985;27:398-401.*18
19. Vorster A, Beighton P, Chetty M, Ganie Y, Henderson B, Honey E, et al. Osteogenesis imperfecta type 3 in South Africa: Cuasative mutations FKBP 102017. South African Med J 2017; 107: 457-462.
20. Cruz-Centeno N, Saenz-Malsonet JF, Lopez-Dones PM, Santiago-Comier A, Oritz-Justiniano VN. Mutation in COL1A1 and COL27A1 associated with a pectus excavatum phenotype in 2 siblings with osteogenesis imperfecta. Am J Case Rep 2022; 23e935526 doi 10.12659/AJCR/.935526.
21. Krassas GE. Idiopathic juvenile osteoporosis. Ann NY Acad Sci 2000; 900: 409-412.
22. Mulpuri K, Slobogean BL, Tredwell SJ. The epidemiology of non-accidental trauma in children. Clin Orthop Relat Res 2011; 469:759-767.
23. Phonela SMH, Goller R, Karsas M. Osteogenesis imperfecta: an overview. South Afr Orthop J 2020;19(4):229-234. dio 10.17159/2309-8309/2020/v19n4a6.
24. Nguyen HTT, Vu DC, Nguyen DM, Dang QD, Tran VK, Le H, Tong SM. Dentinogenesis imperfecta and caries in osteogenesis imperfecta in Vietnamese

children Dental J 2012;9:49. https//doi.org/10.3390/dj9050049.

25. Mohsendaze P, Amirkimi A, Honar N, Saki F, Omrani GHR, Dabbaghmanesh M. Bone density, fractures, and the associated factors in Iranian children and adolescents with osteogenesis imperfecta. BMC Paediatr2021;21:3743.http://doi.org/10.1186/s12887-020-02491-1.
26. Yamaguti P, de La Dure-Molla M, Mannot S, Cardozo-Amaya Y, Baujat G, Michot C, Fournier B, Riou M, Caldas Rosa E, Soares de Lima Y. Unequal impact of COL1A1 and COL1A2 variants on dentinogenesis imperfecta. J Dent Res 2023; 102:616-625.
27. Andersson K, Dahllöf G, Lindahi K, Kindmark A, Grigelioniene G, Astrȍm E, Malmgren B. Mutations in COL1A1 and COL1A2 and dental aberrations in children and adolescents with osteogenesis imperfecta – A retrospective cohort study. PLoS ONE 2017; 12,e0176466.
28. United Referral and Intake System (URIS). Caring for Children with Osteogenesis Imperfecta in Community Program. Manitoba, 2016;1-21.
29. Lingaraju N, Nagarathna PJ, Vijayalakshmi R, Sheshadri P. Osteogenesis imperfecta/Lobstein syndrome associated with osteogenesis imperfecta. J Contemp Dent Pract 2013;14(1):140-142.
30. Barron MJ, McDonnell ST, Mackie I, Dixon MJ. Hereditary dentine disorders: dentinogenesis imperfect and dentine dysplasia. Orphanet J Rare Dis 2008; 3:31 dio: 10.1186/1750-1172-3-31.
31. Beatle ML, Kim JW, Gong SJ, Mudoch-Kinch CA, Simmer JP, Hu JC. Phenotypic variation in dentinogenesis imperfecta/ dentine dysplasia linked 4q21. J Dent Res 2006;84(4):329-333.
32. Akinola R, Disu E, Adewale O. Osteogenesis imperfect: a report of two cases. Internet J Paediatr Neonatol 2008;8(2).
33. Ogundare EO, Akintayo AA, Olatunya OS, Oluwayemi OI, Popoola SO, Atiba AS. Osteogenesis imperfecta – A case series and review of recent advances in management. Ann Health Res 2016;2(2):113-119.
34. Bastos F, Perez LT, de Leȯn Narvȃes CP, Costa O, da Silva RCS, Van-Dunem JCVD, et al. Severe osteogenesis imperfecta: case report. einstein2010;8(4Pt1):480-482.
35. Chein-An S, Chia-Chun L, Yin-Fan C, Jawi-Shan H, Meng-Che T, Yen-Yin C, Chii-Jeng L. et al. Demographic and medical burden of osteogenesis imperfecta: a nationwide database analysis. Osteoporosis International https://doi.org/10.1007/s00198-024-07051-2. Accessed 05/06/2025.
36. Anglan MS, Zaki ME, Hosny L, El-Houssini R, Oteify G, Temtamy SA. Anthropometric measurements in Egyptian patients with osteogenesis imperfecta. Am J Med Genet Part A 2012; 158A: 2714-2718.
37. Lund AM, Muller J, Skovby F. Anthropometry of patients with osteogenesis imperfecta. Arch Dis Child 1999; 80: 524-528.
38. Graff K, Syczewska M. Developmental charts for children with osteogenesis imperfecta type 1 (body height, body weight and BMI). European J Paediatr 2017; 176:311-316.
39. Marini JC, Bordenick S, Heavner G, Rose S, Hintz R, Rosenfield R, Chrousos GP. The growth hormone and somatomedin axis in short children with osteogenesis imperfecta. J Clin Endocrinol Metab 1993; 76:251-256.
40. Stephen LX, Roberts T, Van Hayden E, Chetty M. Osteogenesis imperfecta type III: Psychological challenges. South African Med J 2016; 106:590-593.
41. Wiseman H. The effects of chronic childhood illnesses on families. Nurs Times 1996 Jul 3-9;92(27):44-46.
42. Dogba MJ, Bedos C, Durilgova M, Montpetit K, Wong T, Glorieiux FH. The impact of severe osteogenesis imperfecta on the lives of young patients and their parents – a qualitative analysis. BMC Paedtr 2013; 13:153.

43. Fleck MPA, Leal OF, Louzanda S. Development of the Portugueseversion of OMS evaluation instrument of quality of life (WHOQoL-100) Rev Bras Psiquiatr. 1999;21(1).
44. Marr C, Seasman A, Bishop N. Managing the patient osteogenesis imperfecta: a multidisciplinary approach. J Multidiscip Healthc 2017; 10: 145-155.
45. Marom R, Rabenhorst BM, Morello R. Osteogenesis imperfecta: an update on clinical features and therapies.Eur J Endocrinol 2020; 183: R95-R103.
46. Lozano-Aramburo C, Valazquez-Alvarado M, Villa-Saraza AL, Nunez-Alvarado TP, Lazalde B. The genetic landscape of osteogenesis imperfecta: mutational mechanisms and therapeutic targets. GSC Biol PharmacuetSci 2024; 27(03): 185-194.

SERUM LACTATE DEHYDROGENASE AS A PREDICTOR OF OUTCOMES IN RUSSELL'S VIPER ENVENOMATION

Short Running Title: Serum LDH and outcomes in Russell's viper envenomation

[1,2]AUNG YE KYAW & [2,3]SHYH POH TEO*

1. Department of Medicine, University of Medicine, Mandalay, Myanmar
2. Department of Internal Medicine, RIPAS Hospital, Brunei Darussalam
3. PAPRSB Institute of Health Sciences, Universiti Brunei Darussalam, Brunei Darussalam

*Corresponding Author: shyhpoh.teo@ubd.edu.bn

Submitted: November 2025. Accepted: November 2025

ABSTRACT

Russell's viper (*Daboia russelii*) envenomation is a major cause of morbidity and mortality in Myanmar, often complicated by acute kidney injury (AKI), disseminated intravascular coagulation (DIC), and shock. Serum lactate dehydrogenase (LDH), a marker of tissue injury and hemolysis, shows promise as an indicator of envenomation severity. The objective of this study is to determine the association between serum LDH levels and clinical outcomes in patients with Russell's viper envenomation. A hospital-based cross-sectional study was conducted at Mandalay General Hospital, Myanmar, from January to December 2018. Patients admitted within 72 hours of confirmed Russell's viper bite were included. Serum LDH was measured 18–72 hours after the bite, and outcomes recorded included AKI, DIC, shock, and mortality. Statistical analysis was performed using *t*-tests and one-way ANOVA, with $p < 0.05$ considered significant. Serum samples of 98 patients were analyzed (62.2% male; median age 35 years, range 12–64 years). AKI occurred in 49 (50.0%), DIC in 23 (23.5%), shock in 20 (20.4%) and only 5 (5.1%) patients died. Mean serum LDH was significantly higher among patients with complications: AKI 902.7 vs 389.5 IU/L ($p < 0.001$), shock 1004.5 vs 554.2 IU/L ($p < 0.001$), DIC 1100.0 vs 506.9 IU/L ($p < 0.001$), and death 1146.8 vs 619.2 IU/L ($p = 0.012$). Elevated serum LDH was strongly associated with AKI, DIC, shock, and mortality in Russell's viper bite patients. LDH is an inexpensive and widely available biomarker and is useful for early risk stratification to guide clinical management.

Keywords: acute kidney injury; disseminated intravascular coagulation; envenomation; lactate dehydrogenase; snake bites.

INTRODUCTION:

Snakebite envenomation is a significant public health issue in Myanmar, where the Russell's viper (*Daboia russelii*) accounts for 90% of snake bites, with a case fatality of 8.2% [1]. Complications from Russell's viper bites include acute kidney injury (AKI), disseminated intravascular coagulation (DIC) and shock, which contribute to morbidity and mortality, especially in rural agricultural regions where delayed presentations and limited access to healthcare are common [2,3].

Early identification of patients at risk of severe envenomation is crucial for the timely management and effective use of anti-snake venom (ASV). Clinical parameters, such as prolonged bite-to-injection time and uncoagulable blood status have been associated with poor outcomes [4,5]. However, laboratory markers that can be rapidly and inexpensively measured are needed to improve prognostication. Serum lactate dehydrogenase (LDH), a widely available enzyme marker of tissue injury and hemolysis has been proposed as a potential indicator of envenomation severity [6]. Elevated LDH levels have been associated with hemolysis, rhabdomyolysis and organ dysfunction in viper bite victims [7,8].

This study aimed to evaluate the association between serum LDH levels and clinical outcomes, including AKI, shock, DIC and mortality among patients with Russell's viper bites admitted to Mandalay General Hospital, Myanmar. This hospital is a major tertiary referral center that receives snakebite cases from the Mandalay, Sagaing and Magway regions.

METHODOLOGY

This was a hospital-based cross-sectional study conducted at Mandalay General Hospital, Myanmar from 1st January to 31st December 2018. All patients admitted within 72 hours of a Russell's viper bite were included. Snake identification was based on the description provided by the patient or eyewitnesses, inspection of the dead snake (if available), and/or the presence of clinical features consistent with systemic envenomation.

Patients were excluded if they had pre-existing medical conditions known to elevate serum LDH, such as hemolytic anemia, pancreatitis, chronic hepatitis, lymphoma, leukemia, carcinoma, recent myocardial infarction or pulmonary embolism. Patients who were already presented with AKI, shock or DIC on admission were also excluded.

The following details were obtained from the hospital records:

demographic data, time of the snake bite, time of hospital admission, and time of administration of ASV, as well as clinical manifestations and complications, such as AKI, shock, DIC and mortality. Serum LDH was measured between 18 and 72 hours after viper bite using the kinetic method (ABX Pentra LDH CP reagent, Pentra C400 automatic chemistry analyzer). Serum creatinine was measured daily for the first three days of admission.

The platelet count, the activated partial thromboplastin time (APTT), and prothrombin time (PT) were measured in patients with spontaneous bleeding manifestations.

Acute kidney injury (AKI) was defined as a rise in serum creatinine of at least 1.5 times the baseline or urine output below 0.5mL/kg/hr for at least 6 hours. Patients were considered in shock if the systolic blood pressure was below 80 mm Hg or inotropic support was needed, while DIC was diagnosed in patients with spontaneous bleeding, prolonged PT or APTT and low platelet count.

Data was entered into Microsoft Excel and analyzed using Statistical Program for Social Science (SPSS). Continuous variables were expressed as mean (standard deviation, SD) and categorical variables as frequencies and percentages. Differences in mean serum LDH between two groups were assessed using the independent t-test or among three or more groups using one-way analysis of variance (ANOVA). Normality was assumed based on the sample size and data distribution. A p-value of <0.05 was considered statistically significant.

The study protocol was approved by the Postgraduate Academic Board of the University of Medicine, Mandalay. Patient data were anonymized prior to analysis, and all procedures complied with the principles of the Declaration of Helsinki [9].

RESULTS:

There were 98 patients with confirmed Russell's viper bites, of which 61 (62.2%) were male and 37 (37.8%) were female. The median age of all the patients was 35 years (range 12 to 64 years), with the highest proportion in the 31 to 40 years age group. Most patients were from the Mandalay region (59.2%), followed by Sagaing (37.8%) and Magway (3.1%).

All patients received ASV. The bite-to-needle time for administration of anti-snake venom was within 3 hours for 89 (90.8%),

between 3 and 6 hours in 7 (7.1%) and beyond 6 hours in 2 (2.0%). Nineteen patients (19.4%) received less than ten vials of ASV, 33 (33.7%) received 10 to 20 vials, 23 (23.5%) received 20 to 30 vials, and 23 (23.5%) required more than 30 vials. The mean total volume of ASV used per patient was 201 mL, increasing to 272 mL among patients who died.

Among the 98 patients, 42 (42.9%) had no complications, 26 (26.5%) developed one complication, while 30 (30.6%) had two or more complications. AKI occurred in 49 (50.0%), shock in 20 (20.4%), DIC in 23 (23.5%), while mortality occurred in 5 (5.1%) patients.

Serum LDH was measured in 91 patients; 56 (61.5%) had LDH levels above the normal range. LDH was significantly higher in patients with AKI (902.7±72.5 IU/L) than those without AKI (389.5±28.4 IU/L, $p < 0.001$). Patients with shock had a higher mean serum LDH (1004.5 ± 63.8 IU/L) compared to those without shock (554.2 ± 51.5 IU/L, $p<0.001$). Patients with DIC also had a higher mean serum LDH (1100.0 ± 137.5 IU/L) compared to those without DIC (506.9 ± 29.8 IU/L, $p < 0.001$). LDH increased progressively with the number of complications, where mean serum LDH was 331.9 ± 128.8 IU/L in patients with no complication, 651.2 ± 159.1 IU/L with one complication and 1081.5 ± 573.5 IU/L with two or more complications ($p < 0.001$, ANOVA). Patients who died also had a significantly higher LDH (1146.8 ± 148.9 IU/L) compared to 619.2 ± 47.0 IU/L ($p = 0.012$) in survivors.

DISCUSSION:

This study evaluated the association between serum LDH levels and clinical outcomes among patients with Russell's viper envenomation admitted to Mandalay General Hospital. LDH levels were significantly higher in patients with AKI, shock, DIC and mortality, compared to patients without these complications. The progressive rise in LDH with increasing number of complications also adds weight to support its role as a biochemical marker of disease severity. These findings are consistent with studies showing that elevated LDH reflects tissue injury, hemolysis, and rhabdomyolysis following viper envenomation [7,8, 10].

The mechanisms for these complications include phospholipase A2-mediated cellular damage, coagulopathy, and microangiopathic hemolysis, which has been described in detail previously [6]. LDH elevation likely represents a composite effect of direct venom toxicity and

secondary complications such as DIC and renal ischemia. As LDH rises within 18 hours of cell injury after Russell's viper bite, and remains elevated for several days, it offers a practical time window for clinical monitoring during the early phase of hospitalization.

The proportion of patients who developed AKI (50%), DIC (23.5%) and shock (20.4%) are similar to previous reported cases from Myanmar [11,12]. However, the mortality rate in this study was lower (5.1%) compared to the other studies, which exceeded 10% [11,12]. This may reflect improved ASV availability in this tertiary center. In addition, patients with delayed treatment beyond three hours, or requiring higher ASV doses tended to experience more severe complications. These findings support the continuing importance of timely anti-venom administration and supportive care.

From a clinical perspective, LDH is an inexpensive and widely available assay in Myanmar. Routine LDH measurement may aid early identification of patients at risk of systemic envenomation and guide triage decisions, especially where advanced laboratories or imaging facilities are unavailable. However, LDH is non-specific and may also be elevated in conditions such as myocardial infarction, sepsis or hemolytic anemia [13]. Thus, interpretation should be contextual and combined with clinical assessment and other laboratory indices.

The main limitations are that the study was conducted in a single tertiary center and may over-represent severe referred cases, leading to selection bias. The cross-sectional design limits causal inference, and LDH trends over time were also not measured. Future prospective studies with serial LDH measurements may help validate its prognostic utility.

CONCLUSION

Serum LDH showed a strong association with AKI, DIC, shock, and mortality in Russell's viper bite patients. As LDH is an inexpensive and widely available biomarker, LDH shows potential as a useful adjunct in assessing envenomation severity and guiding management in snake bite patients.

Acknowledgements

We would like to acknowledge Professor Khin Mg Lwin, Professor Yan Lynn Myint, Professor Win May Thein, Professor San San Hlaing, Professor Soe Moe Naing, Dr May Po Po Thar and Dr Nang Mei Mei Tun from Mandalay University of Medicine and Mandalay General Hospital, Myanmar for

their advice and support to carry out this study.

REFERENCES

1. White J, Alfred S, Bates D, Mahmood MA, Warrell D, Cumming R, Thwin KT, Thein MM, Thant M, Naung ZM, Naing YH, San SS, New MT, Peh CA. Twelve-month prospective study of snakebite in a major teaching hospital in Mandalay, Myanmar; Myanmar Snakebite Project (MSP). Toxicon: X 2019; 1:100002.
2. Hsieh YH, Hsueh JH, Liu WC, Yang KC, Hsu KC, Lin CT, Ho YY, Chen LW. Contributing factors for complications and outcomes in patients with snakebite; experience in a medical center in Southern Taiwan. Ann Plastic Surgery 2017;78(3): S32-S36.
3. Warrell DA. Snake bite. Lancet 2010;375(9708):77-88.
4. Lu Q, Clemetson JM, Clemetson KJ. Snake venoms and hemostasis. J Thromb Haemost 2005; 3(8): 1791-1799.
5. Maduwage K, Isbister GK. Current treatment for venom-induced consumption coagulopathy resulting from snakebite. PLoS Negl Trop Dis 2014;8(10): e3220.
6. Kyaw AY, Teo SP. Review of pathophysiology of Russell's viper envenomation and clinical utility of serum lactate dehydrogenase as a predictor of severity and outcome. Pac J Med Sci 2025;26(2):41-48.
7. Bhagwat K, Amar L. Blood hemoglobin, lactate dehydrogenase and total creatine kinase combinely as markers of hemolysis and rhabdomyolysis associated with snake bite. Int Journal of Toxicol Pharm Res 2013;5(1):5-8.
8. Kandasamy S, Gopalakrishnan S, Venkatesan M, Ramakrishnan M. The clinical and biochemical profile of snakebite patients – a hospital based comparative study of envenomed and nonenvenomed victims. Int J Biochem Biotechnol 2014;3(2):511-515.
9. World Medical Association. WMA Declaration of Helsinki – Ethical principles for medical research involving human participants [Internet]. WMA, 2024. [updated 2024 Oct, cited 2025 Nov 17]. Available from: https://www.wma.net/policies-post/wma-declaration-of-helsinki/
10. Patil SL, Kaveri VB. To study the utility of serum LDH values as a marker of hemotoxicity in snake bite victims in HSK hospital: an observational study. Indian J Basic Appl Med Res 2017;6(2):331-347.
11. Myint AA, Myint TL, Pe T. Poisonous snakebites of Myanmar with special references to the bites and case fatality rate (1998-2005). Myanmar Health Sci Res J 2007;19(2):87-93.
12. Pe T, Myint AA, Kuy KA. Russell's viper (Daboia russelii siamensis) bite: venom and antivenom clearance and outcome of antivenom therapy. Myanmar Health Sci Res J 2006;18(2):67-72.
13. Adams MJ, Buehner M, Chandrasekhar K, Ford GC, Hackert ML, Liljas A, Rossmann MG, Smiley IE, Allison WS, Everse J, Kaplan NO, Taylor SS. Structure – function relationships in lactate dehydrogenase. Proceedings Natl Acad Sciences USA 1973; 70(7): 1968-1972.

CANNABIS SATIVA EXACERBATES INFLAMMATORY RESPONSES IN MALE AND FEMALE WISTAR RATS

Running title: Effect of *Cannabis sativa* on inflammatory markers in Wistar Rats

[1]**OLABISI ELIZABETH AYOOLA,** [2,3*]**AMUDA OLUWASOLA,** [4]**GARBA SA'ADU**

1. Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Kwara State University, Malete, Nigeria.
2. Department of Physiology, Faculty of Basic Medical Sciences, University of Ilesa, Ilesa, Osun State, Nigeria.
3. Department of Nursing Science, Physiology Unit, Faculty of Allied Health Sciences, Jimoh Babalola University, Ilorin, Kwara State, Nigeria
4. Ahmadu Bello University, Zaria, Kaduna State, Nigeria

*Corresponding author: dr.amuda19@gmail.com

Submitted: November 2025. Accepted: December 2025

ABSTRACT:
Consumption of *Cannabis sativa* (CS) (Marijuana) has been known to be a psychoactive substance which has deleterious effects on the body cells. This study was conducted to investigate the inflammatory responses in male and female Wistar rats following administration of CS. Twenty male (m) and twenty female (f) rats were separately assigned into four groups of five animals each. The rats in groups 1m & If, 2m & 2f, 3m & 3f and 4m & 4f received orally 1mL of distilled water (control), 2mg/kg body weight (bw) of CS, 4mg/kgbw of CS and 6mg/kgbw of CS respectively for twenty-one (21) days. Inflammatory markers (C-reactive protein (CRP), Tumor necrosis factor (TNF), Interleukin-6 (IL-6), Myeloperoxidase (MPO), and Nitric oxide (NO)) were quantified using standard procedures. There was no significant ($p>0.05$) difference in CRP, TNF, IL-6, MPO, and NO in the groups treated with low dose of CS (2mg) but with significant ($p<0.05$) increase in high doses (4mg and 6mg) groups when compared with the control in both male and female rats. This study showed that CS stimulated inflammatory responses due to increase in CRP, TNF, IL-6, MPO, and NO levels compared to that of the control. However, this effect was dose-dependent, and it was more in male than female rats.

Keywords: *Cannabis sativa*, Inflammatory markers, Dose-dependent, Wistar rat
Submitted: November 2025, Accepted: December 2025

INTRODUCTION:

Cannabis sativa (CS) is commonly referred to as Marijuana, dope, pot, grass, weed, head, MaryJane, doobie, bud, ganja, hashish, hash, and bhang and has long been used in folk medicine. It is anxiolytic, sedative, analgesic and psychedelic. About 3.9% of the world's population used CS, according to the World Drug Report [1]. Cannabis derivatives, such as delta-9-tetrahydrocannabinol (delta-9-THC) and cannabidiol (CBD), are involved in several neurotransmitter systems, such as glutamatergic, serotonergic, noradrenergic, and dopaminergic neurons [2]. These neurotransmitters are responsible for the therapeutic and recreational effects of cannabis. Inflammation is the body's innate response to injury or insult, including infection, trauma, surgery, burns, and cancer. Cytokines are a small group of proteins released into the bloodstream during inflammation. If their concentrations increase or decrease by at least 25%, they can be used as systemic inflammatory markers [3-6]. The inflammatory markers, also known as acute phase reactants, that are most measured in clinical practice are C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), interleukin-10, interleukin-6, and tumor necrosis factor (TNF). Several research works have been done on effects of cannabis on inflammatory markers both in human and animals [3-6]. However, none of these research works has explored gender differences as well as variations in the dosages of CS. This research work, therefore, aims to bridge these lacunae by investigating the effects of CS on both male and female Wistar rats to identify potential sex differences and examine specific inflammatory markers (CRP, ESR, IL-6, TNT and NO), including their dose-dependent effects.

METHODOLOGY:

Sample collection:

Cannabis sativa (CS) leaves were donated by the National Drug and Law Enforcement Agency (NDLEA), Nigeria, for research purpose only.

Extraction of *Cannabis sativa* leaves

Extraction of *Cannabis sativa* (CS) was done with Soxhlet apparatus by soaking 600 grams of CS in 98% ethanol for 48 hours. It was filtered and the filtrate was poured into a round-boom conical flask it was fixed with a rotary evaporator. The filtrate was then evaporated and cooled. The dried yield of the extract was 60g (weight of the extract obtained after drying).

Experimental animals

Twenty male rats with mean weight of 160 ± 1.12g and twenty female rats with mean weight of 125 ± 1.35gused in the present study were obtained from Temilade Animal Venture,

Ogbomoso, Oyo State, Nigeria. The animals were housed at room temperature with unrestricted access to diet and water and maintained on a daily light/dark cycle. Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed. The experimental protocol was approved by Ethical Committee of the Jimoh Babalola University, Ilorin, Kwara State, Nigeria with approval number (JBU/ERC/2025/05).

Experimental protocol:

After 2 weeks of acclimatisation, the animals (male (m) and female (f)) were separately and randomly assigned into four groups of five animals each for male and female.

The rats in groups 1m & 1f, 2m & 2f, 3m & 3f and 4m & 4f received orally (in the morning) 1.0 mL of distilled water (control), 2.0 mg/kg body weight (bw) of CS, 4.0 mg/kg bw of CS and 6.0 mg/kg bw of CS respectively, for twenty-one days. The animals had access to food and water ad-libitum. They were sacrificed after day 21.

Preparation of serum:

The male and female rats were sacrificed under ketamine anesthesia and blood was collected by cardiac puncture into sample bottles. The blood was left for 30 min to clot and thereafter centrifuged at 625×g for 10 min using a Uniscope Laboratory Centrifuge (Model SM800B, Surgifield Medicals, Essex, England). The serum was collected into plain bottles with the aid of a Pasteur pipette. Sera were stored in a freezer maintained at -5 °C and used within 12 hours of preparation.

Assay kits:

The Inflammatory markers (TNF, IL-6, NO, MPO, AND CRP) were quantified according to the instruction provided by assay kit manufacturers, using microplate immunoenzymometric (EMA/ELISA) assays [7]. The serum inflammatory markers concentrations were then interpolated from their respective calibration curves. The analyzer was calibrated and validated for use with rat sera. All the assay kits used were products of Monobind Inc., Lake Forest, California, USA. All other chemicals used were products of Sigma Aldrich Company, Mannheim, Germany.

Statistical analysis:

Results were expressed as the mean ± standard error of mean (S.E.M).

Data was analyzed using a two-way Analysis of Variance, followed by the LSD post-hoc test to determine significant differences in all the parameters with the aid of graph pad, version 9.0. Differences with values of $P<0.05$ were considered statistically significant.

RESULTS:

Table 1: Showing inflammatory markers of rats (male and female) for control and treated (2.0 mg/kg bw, 4.0 mg/kg bw and 6.0 mg/kg bw) groups.

	Interleukin-6 (pg/ml)	Tumour necrosis factor (ng/ml)	C-reactive protein	Nitric oxide (Um)	Myeloperoxidase (U/l)
Control (m)	0.3±0.012	14.0±0.321	18.0±0.010	3.0±0.112	0.40±0.041
Control (f)	0.1±0.005	9.0±0.270	10±0.341	1.0±.0.051	0.22±0.070
2mg/kg bw CS(m)	0.7±0.021	35.0±1.042	45.0±1.123	5.0±0.250	0.58±0.023
2mg/kg bw CS (f)	0.3±0.012	18.0±0.385	28±1.014	2.0±0.030	0.42±0.050
4mg/kg bw CS (m)	1.2±0.041 [a]	48.0±1.230 [a]	55.0±0.980[a]	9.0±0.741 [a]	0.81±0.037 [a]
4mg/kg bw CS (f)	0.6±0.032[a b]	30.0±0.973 [a b]	40.0±0.778 [a b]	5.0±0.055 [a b]	0.55±0.043 [a b]
6mg/kg bw CS (m)	1.8±0.022 a	60.0±1.721a	75.0±1.312a	12.0±1.027a	0.97±0.008 a
6mg/kg bw CS (f)	1.4±0.017 [a b]	42.0±1.069 [a b]	58.0±1.070 [a b]	7.0±0.084 [a b]	0.73±0.016 [a b]

Note: aP<0.05 vs control and 2.0 mg/kg bw (m and f); bP<0.05 vs (4.0 and 6.0) mg/kg bw male.

The results obtained are presented in Table 1. There was no significant (p>0.05) difference in CRP, TNF, IL-6, MPO, and NO levels in the groups treated with low dose (2.0 mg) of CS when compared with the control. However, there were significant (p<0.05) increases at high doses (4.0 mg and 6.0 mg) of CS when compared with the control. In addition, there were significance (p<0.05) differences in CRP, TNF, IL-6, MPO, and NO levels in the groups treated with high doses between male and female rats.

DISCUSSION:

There exists a tight integration between the immune and nervous systems, the so-called inflammatory reflex, capable of influencing both systems in response to inflammatory and infectious agitation of homeostasis [8]. Essentially, the autonomic nervous system is implicated in a bidirectional inflammatory reflex with the vagal nerve being the main neuronal substrate of an immunoregulatory role, providing a fast and subconscious anti-inflammatory response [9]. Given their important role, many experts have speculated that CS plays a role on inflammatory markers. Several studies have shown that the effects of CS on various physiological processes are dose-dependent [10-18]. Although research implicating the role of inflammatory markers is still evolving, there is some evidence for the role of Inflammatory markers, such as interleukin-6, Nitric oxide, tumour necrosis factor, myeloperoxidase and C-Reactive protein in the body. These inflammatory markers are implicated in

inflammation of the body more broadly and may be activated through injury, burn or infection. For example, a physical condition in which part of the body becomes reddened, swollen, hot and often painful.

Our findings revealed that CS (at high doses) stimulated inflammatory responses by increasing the levels of TNF, IL-6, NO, MPO, AND CRP in the animals. This could be due to tissue injury caused by CS which was consistent with the finding of Wang et al [20]. However, no changes in the levels of these inflammatory markers were observed at low doses, suggesting that the mechanisms of action of CS-receptors are dose-dependent. Additionally, the effect of CS on inflammatory markers were more in male than in female. This may be attributed to sex-dependent differences in cannabis metabolism and interactions between the endocannabinoid system and sex hormones which could prone females to be more sensitive to the behavioral and physiological effects of cannabis than males [18]. Another factor could be through cannabinoid receptor expression and signaling pathways.

Cannabinoid receptors are more widely distributed in males than females [21].

CONCLUSION:

This study showed that CS could cause toxicity on inflammatory markers which could be mediated by causing tissue injuries. However, these effects were dose dependent. This study concluded that consumption of CS at high doses could impose serious threat on inflammatory markers in both sexes.

Acknowledgement

We appreciate National Drug Law Enforcement Agency (NDLEA), Nigeria, for their kind donation of the quantity of CS leaves used for this study. We also acknowledged Dr. Emeka for the laboratory analysis of this research work. This research work did not receive any financial support but was solely financed by the authors.

REFERENCE:

1. Canton H. United Nations Office on drugs and crime—UNODC. In the Europa Directory of International Organizations. Vol. 28, July 2021:240-244.
2. Malabadi RB, Sadiya M, Kolkar KP, Lavanya L, Chalannavar RK. Quantification of THC levels in different varieties of Cannabis sativa. International Journal of Science and Research Archive. Vol. 10(2), May 2023:860-73.
3. Hopkins S, Kelley T, Roller R, Thompson RS, Colagiovanni DB, Chupka K, Fleshner M. Oral CBD-rich hemp extract modulates sterile inflammation in female and male rats. Frontiers in Physiology. Vol.14 May 2023:1112906.
4. Lima MG, Tardelli VS, Brietzke E, Fidalgo TM. Cannabis and inflammatory mediators. European Addiction Research. Vol. 27(1), July2021 29:16-24.
5. Okafor CN, Li M, Paltzer J. Self-reported cannabis use and biomarkers of inflammation among adults in the United States. Brain, behavior, & immunity-health. Vol. 7, August 2020:100109.
6. Alshaarawy O, Sidney S, Auer R, Green D, Soliman EZ, Goff Jr DC, Anthony JC. Cannabis use and markers of systemic

inflammation: the coronary artery risk development in young adult's study. The American journal of medicine. Vol. 132(11), November 2019:1327-34.
7. Leng SX, McElhaney JE, Walston JD, Xie D, Fedarko NS, Kuchel GA. ELISA and multiplex technologies for cytokine measurement in inflammation and aging research. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. Vol. 63(8), Aug 2008: 879-84.
8. Dantzer R. Neuroimmune interactions: from the brain to the immune system and vice versa. Physiological reviews. Dec 2017.
9. Williams DP, Koenig J, Carnevali L, Sgoifo A, Jarczok MN, Sternberg EM, Thayer JF. Heart rate variability and inflammation: a meta-analysis of human studies. Brain, behavior, and immunity. Vol. 80, August 2019:219-26.
10. Oluwasola A, Lukman J, Comfort AM. Effect of Cannabis sativa on Haematological Parameters in Male and Female Wistar Rats. Asian Pacific Journal of Medical Toxicology. Vol. 13(3), November 2024: 90-94
11. Oluwasola A, Usman, LN. Oxidative stress Markers of Male Wistar Rats following recovery period from exposure to Cannabis sativa. Pacific Journal of Medical Sciences. Vol. 25(2), August2024: 65-74.
12. Oluwasola A, Kolawole Y., Usman, LN, Olanrewaju OA. Effect of co-administration of Cannabis sativa and Vitamin C on Biomarkers of Oxidative stress in Female Wistar Rats. Pacific Journal of Medical Sciences. Vol. 25(2), August 2024: 57-64.
13. Oluwasola A, Ayoola OE, Garba S, Adepoju MA, Biliaminu SA, Olayaki LA. Melatonin Mitigates Hormonal Toxicity in Cannabis - Treated Female Wistar Rats. The Tropical Journal of Health Sciences. Vol. 30(2), July 2023 5: 14-19
14. Oluwasola A, Ayoola OE, Odetayo AF, Garba S, Olayaki LA. Ameliorative Effect of Melatonin on Reproductive Hormones in Ethanol Extracts of Cannabis sativa-Treated Female Wistar Rats. NISEB journal. Vol. 22(2), December 2022: 27-32.
15. Oluwasola A, Olayaki LA, Ayinde TO. Effects of Melatonin and Ethanolic Extract of Cannabis sativa Leaves on Sexual Behaviour Parameters of Female Wistar Rats. Bioscience Research Journal. Vol. 32(1), Feb 2020: 29-36.
16. Olayaki LA. Oluwasola A. Melatonin Mitigates Ovarian Toxicity in Cannabis-Treated Female Witar Rats. Federation of American Societies for ExperimentalBiology Journal. Vol. 34(S1),March 2020 19:1-1.
17. Oluwasola A, Olayaki LA, Ayinde TO. Effects of Melatonin on Estrous Cycle Changes Induced by Etanolic Extract of Cannabis sativa in Female Wistar Rats. Nigeria Society of Experimental Biology Journal. Vol. 19(12),June 2019 13:55-60
18. Oluwasola A, Olayaki LA, Ayinde TO. Melatonin Mitigates Oxidative Stress in Ethanolic Extract of Cannabis-Treated Female Wistar Rats. Nigeria Journal of Biochemistry and Molecular Biology. Vol. 35(1),February2019 8:7-16.
19. Wang X, Lin C, Jin S, Wang Y, Peng Y, Wang X. Cannabidiol alleviates neuroinflammation and attenuates neuropathic pain via targeting FKBP5. Brain, behavior, and immunity. Vol. 111, July 2023:365-75.
20. Cooper ZD, Craft RM. Sex-dependent effects of cannabis and cannabinoids: a translational perspective. Neuropsychopharmacology. Vol. 43(1), January 2018:34-51.
21. Liu X, Li X, Zhao G, Wang F, Wang L. Sexual dimorphic distribution of cannabinoid 1 receptor mRNA in adult C57BL/6J mice. Journal of Comparative Neurology. Volume 528 (12), August 2020:1986-99.

PROPOSED PROTOCOL FOR DEVELOPING AND INTEGRATING COMMUNICATION SKILLS CURRICULA FOR UNDERGRADUATE PROGRAMS AT THE PAPUA NEW GUINEA UNIVERSITY OF MEDICINE AND HEALTH SCIENCES

ETUPARO A. BUKA

Division of Public Health, School of Medicine and Health Sciences
University of Papua New Papua New Guinea

Correspondence to: ebuka@upng.ac.pg / eazavu@gmail.com

Submitted: November 2025: Accepted: December 2025

ABSTRACT

The University of Papua New Guinea's School of Medicine and Health Sciences (SMHS) is transitioning into a stand-alone university. A new academic program proposed for immediate implementation is the Medical and Health Sciences Foundation Year with Communication Skills as one of the courses. Communication skills is a cross-cutting component taught throughout the undergraduate medical program. Other undergraduate programs are yet to develop communication skills as a core curriculum. This protocol is to guide the development of a core communications curriculum for each program in the PNGUMHS. Experts, stakeholders and faculty, from each program are identified to participate in the curriculum development process which is split into five phases. Phase 1 involves assessment and literature review conducted by faculty to assess current state of communication education in each program. Literature reviews focus on various instruments used in developing, integrating, teaching and assessment, including analysis of existing communication curricula and identifying communication competencies expected of graduates. Phase 2 involves identifying internal and external experts and stakeholders to participate in curriculum development. In Phase 3, information collected is synthesized to develop draft curricula for each program. Phase 4 involves the Delphi technique to make refinements and finalize the curricula. Phase 5 involves implementation and evaluation of each curriculum. The PNG National Human Resources for Health Strategic Plan 2021-2030 has identified, among others, two relevant challenges. 'The competency of health workers not aligned to population and health service needs' and 'curriculum for health professions training is not standardized.' Therefore, the finalized curricula are anticipated to serve as models in achieving competency-based standardized communication skills curricula for each undergraduate program.

Key Words: Communication skills curriculum, undergraduate health programs, core communication curricula, curriculum development process

INTRODUCTION:

The School of Medicine and Health Sciences (SMHS) of the University of Papua New Guinea (UPNG) is the country's leading higher education institution in the education and training of different cadres of health care professionals, - medical laboratory scientists, pharmacists, dental technologists, nurses, and medical doctors. The SMHS is currently in the transition phase to becoming a stand-alone university – The Papua New Guinea University of Medicine and Health Sciences (PNG UMHS). As such, one of the new academic programs proposed for immediate implementation is the Medical and Health Sciences Foundation Year (MHSFY) with Communication Skills as one of the courses. The MHSFY is designed to provide a pathway for students who want to pursue careers in the different health professions. It aims to equip students with the foundational knowledge and skills needed for advanced studies in medicine and other health-related degree programs. Successful completion of the program will allow students to progress to relevant degree programs in the PNG UMHS.

Communication skills is a cross-cutting component that is taught under the Professional Skills Domain (course) of the undergraduate MBBS program unlike the other undergraduate programs.

The communication skills taught are often related to reading and writing skills in which students apply the skills in writing essays, reading research articles and writing literature reviews, writing research proposals and reports or writing laboratory reports which are also essential skills in health care practice.

However, a review of the literature indicates that there is great importance placed on patient centeredness in health care practice [1, 2]. Patient-centered care necessitates that communication skills be integrated throughout the undergraduate curricula for all health professions programs at all levels and not just at the foundation year level. While foundational communication skills are important, ongoing practice and refinement throughout the curriculum are necessary to address the diverse communication needs of patients and various healthcare settings [1-2].

Hence, the creation of a new Communication and Life Skills course for the new MHSFY program provides an opportunity to develop core communication skills curricula for each undergraduate program. This is a valuable and increasingly recognized opportunity for the new PNG UMHS to contribute in a significant way to the development of well-rounded, competent and compassionate health care professionals.

Furthermore, the new university is expected to meet the national standards for institutional registration that are stipulated in the PNG National Standards for Higher Education

Institutional Registration for PNG Higher Education Institutions, 2024 [3]. The specific standard relevant for this discussion is Standard 6: 'The Institution's Programs, Teaching and Assessment are Adequate to Achieve the Expected Student Learning Outcomes' with special focus on the Curriculum Development Policy [3]. This policy requires higher education institutions to outline comprehensive procedures for the design, review, and revision of academic programs to ensure alignment with the PNG National Qualifications Framework (PNGNQF), stated learning outcomes, industry standards, and emerging trends in the field [3]. The PNGNQF outlines stringent operational procedures for programming and institutional accreditation that the MHSFY program needs to follow [4].

METHODOLOGY

To develop communication curricula for the undergraduate programs, a systematic academic approach and the Delphi technique is used. The Delphi technique is a systematic iterative method for gathering and refining expert opinions to achieve consensus on specific issues [5].

Achieving consensus on communication curricula for the undergraduate programs is vital because it ensures broad agreement among experts on essential communication skills or skills needed by graduates from each health care profession in PNG. Furthermore, involving experts from each health profession helps create a standardized curriculum that can be adapted and implemented across each program, not only at the new PNG UMHS but also at other higher education institutions offering the same programs. This reduces variability in training and ensures that all students receive a consistent foundation in communication skills. The input of diverse experts from various health professions also ensures that communication curricula are evidence-based, relevant, consistent and collaborative, ultimately contributing to better patient care and improved healthcare outcomes [5 – 7].

The curriculum development process is split into five (5) phases.

Phase 1: Needs assessment and review of literature:

The assessment is to identify gaps in the current curriculum and determine the specific communication skills needed by future health professionals [8, 9]. It involves defining the specific undergraduate programs for which the curriculum is intended, and which health professions will be involved in the curriculum development [10].

A total of 12 undergraduate programs are currently offered by the SMHS [11]. Faculty members will identify areas for improvement and integration of communication skills into their respective programs such as specific

communication competencies expected of graduates, exploring different curriculum models used in integrating communication skills into the curriculum such as horizontal integration (across different courses) and vertical integration (across different years); examining various methods used in assessing communication skills and examining how integrated curricula contribute to the development of well-rounded healthcare professionals with strong interpersonal skills [12 – 14].

Phase 2: Expert and Stakeholder Consultation and Input:

Both internal and external experts and stakeholders are identified and invited to take part in curriculum development. Internal experts and stakeholders include faculty with expertise in teaching communication in healthcare and administrators within the university. External experts and stakeholders include practicing clinicians, practitioners from other health care professions, community members, professional organizations, employers and regulatory bodies such as the PNG Medical Board that approves practitioner licenses. Understanding expert and stakeholder perspectives on communication needs and expectations and using this information to inform curriculum development can ensure that communication curricula are relevant, effective, and prepare students to meet the diverse communication demands of healthcare practice. The stakeholder engagement methods include mainly surveys and interviews to gather diverse perspectives [15 – 17].

Phase 3: Synthesizing Information obtained from Phases 1 and 2.

The information collected are synthesized to identify the specific communication skills that are most relevant and align them with the learning outcomes for each program; select and adapt teaching methods that are effective in promoting communication skills development; choose assessment methods that are appropriate for evaluating the identified learning outcomes and align them with the chosen teaching strategies and tailor the curriculum to the specific context of each program taking into account the program's goals, resources, and the needs of the students and faculty. An initial draft of the communication curricula is developed for each program, to be further refined after the Delphi study [18, 19].

Phase 4: Delphi Technique:

The Delphi technique is a structured approach that provides a systematic and organized way of gathering and refining expert and stakeholder opinions through multiple rounds.

Expert and Stakeholder Panel Selection:

The same experts and stakeholders from phase 2 are invited again to review their suggested communication competencies that graduates should have acquired upon graduating from each program.

Snowball techniques are also used to ask participants if they know of anyone else with expertise in health communication who could be invited to review the draft competencies and provide more inputs [20 – 23].

Subsequent Rounds (Refinement):

Facilitators analyze the responses and create a summary of key communication competencies and present it to the participants in subsequent rounds to rate or rank the importance of the different competencies, using Likert scales or other quantitative methods. Participants are also given the opportunity to provide further comments and justify their ratings. The process is iterative, with the facilitator providing feedback on the group's responses in each round and refining the competencies based on the consensus or divergence of opinions [20 – 23].

Reaching Consensus or Defining Divergence:

The Delphi technique aims to reach a consensus among experts, but it can also be used to identify and understand divergent opinions. Consensus is typically defined by a pre-determined level of agreement which is 70% or 80% on the competencies.

This study will aim to reach the required level of agreement. However, if consensus is not fully achieved, the process helps to clarify the range of expert and stakeholder opinions on the competencies so they can still be used to inform the design of the curriculum [20 – 23].

Curriculum Design:

The final set of communication competencies, whether representing consensus or a range of views, is used to inform the design of the communication curriculum. The draft curricula developed in phase 3 for each program are reviewed and finalized for implementation.

The main focus areas for review include learning objectives (outcomes), content areas, teaching methods, and assessment strategies. The curricula are then integrated into the respective existing undergraduate programs, ensuring they are aligned with other relevant courses and clinical experiences [20 – 23].

Phase 5: Implementation and On-Going Refinement:

The integrated curricula for each program are implemented and evaluated to determine their effectiveness through ongoing assessment and feedback. The on-going assessment and refinement of the curricula will be done every three to five years based on information collected through the following feedback mechanisms to ensure they remain relevant and effective.

Student feedback: Faculty involved in teaching communication collect and analyze student feedback to identify areas for improvement and ensure the curriculum remains relevant to students' needs [24].

Faculty Input: Faculty members who teach communication skills provide insights into the challenges and successes of the integrated curriculum [25].

Expert and Stakeholder Input: Practicing healthcare professionals, including the experts and stakeholders who participated in the curriculum development process, are invited to provide feedback on the communication skills required in real-world settings. Their input will help align the curriculum with current best practices and address gaps in training.

Incorporating new research findings: New research findings are incorporated and the curriculum adapted to address emerging challenges and communication needs in healthcare [7].

DISCUSSION:

Communication skills of the different cadres of health care professionals can be cultivated and honed through education and training. Communication skills courses can be introduced in undergraduate health education programs and taught through the entire duration of the programs to assess continuous demonstrated improvements in students' communication skills. Internationally, the teaching and assessing of interpersonal and communication skills are now accepted as an integral component of medical and related health education programs [26].

For example, there is widespread acceptance of behavioral sciences and in particular, communication skills as an important component of dental education in the UK, US, Canada and New Zealand. In the United States, communications skills training is an important cornerstone of dental education [27].

In India, most dental schools are reported to have added health communication in their curricula which helps in teaching and assessing competencies in communication skills among dental students [28].

Across Europe, 121 communication experts from 15 professional fields and 16 European countries participated in developing a health professions core communication curriculum (HPCCC) framework for all undergraduate European health care education through a consensus process [7]. This framework is currently used as a guide for teaching communication inter- and multi-professionally in undergraduate education in health care across Europe [7]. These and many similar initiatives undertaken by other countries are often in response to policy directives from either their national governments or international organizations such as the World Health Organization. One such document is the *WHO Global Strategy on Human Resources for Health: Workforce 2030* which emphasizes the critical role of education and training in strengthening the health workforce to meet current and future health system needs [29].

A policy option suggested for WHO member states to consider is the *'adoption of transformative strategies in the scale-up of health worker education'* which requires a coordinated approach to link human resources for health (HRH) planning and education and encourage inter-professional education and collaborative practice [29]. In response to this policy option, Papua New Guinea's National Department of Health (NDOH), an Island WHO member state in the Western Pacific region has developed a national document titled *National Human Resources for Health (HRH) Strategic Plan 2021-2030* [30].

The Plan has six (6) strategic objectives with strategic challenges and proposed interventions clearly outlined for each strategic objective. The strategic objective relevant for this discussion is *objective 5: improve the capacity of the education institutions to produce competent health professions*. This strategic objective focuses on the need to up-scale the production of competent health workforce by adopting a common competency-based education framework to ensure that graduates are fit for practice. It also focuses on improving efficiency in instructional design and teaching, allow implementation across the nation, facilitate curriculum assessment and allow response to change. Among other strategic challenges identified under this strategic objective, two relevant challenges for this discussion are *'competency of health workers not aligned to population and health service needs'* and *'curriculum for health professions training are not standardized* [30]. The finalized curricula developed from implementing this proposed protocol are anticipated to serve as models in achieving competency-based standardized communication skills curricula for each undergraduate program at the new PNG UMHS.

CONCLUSION:

A meticulously planned and coordinated collaboration with key experts and stakeholders and the new university in planning, developing, implementing and evaluating health workforce education and training programs ensures that the national standards and procedures are met. Compliance with national policies and procedures ensures quality assurance of the academic programs which in turn guarantees quality educational and training outputs for the local health workforce needs [5].

The proposed protocol assures comprehensiveness of an academic program which is a key factor in attracting students who seek a well-rounded and robust academic experience. It can also attract a diverse and talented faculty, leading to a more vibrant and engaging learning environment. Attracting students and talented faculty are crucial factors in a highly competitive higher education landscape [5].

REFERENCE:

1. Cakmak C, Ugurluoglu O. The effect of patient-centered communication on patient engagement, health-related quality of life, service quality perception and patient satisfaction in patients with cancer: a cross-sectional study in Turkiye. Cancer Control. 2024. doi: 10.1177/10732748241236327
2. Jenerette CM, Mayer DK. Patient-provider communication: the rise of patient engagement. Semin Oncol Nurs). 2016; 32(2):134-143. https://doi.org/10.1016/j.soncn.2016.0.
3. Department of Higher Education, Research, Science and Technology (DHERST). Papua New Guinea National Standards for Higher Educational Institution Registration. Policy Document. 2024. Port Moresby. Papua New Guinea.
4. Commission for Higher Education (CHE). 2010. Papua New Guinea National Qualifications Framework. Policy Document. 2010. Port Moresby. PNG
5. De Villiers MR, Pierre J.T. de Villiers PJT, Kent AP. The Delphi technique in health sciences education. Med. Teach. 2005;27(7):639-643. doi: 10.1080/13611260500069947
6. Fink A, Kosecoff J, Chassin M, Brook RH. Consensus methods: characteristics and guidelines for use. RAND Publications. https://www.rand.org/content/dam/rand/pubs/notes/2007/N3367.pdf
7. Bachmann C, Abramovitch H, Barbu CG, Cavaco AM, Elorza RD, Haak R, Loureiro E, Ratajska A, Silverman J, Winterburn S, Rosenbaum M. A European consensus on learning objectives for a core communication curriculum in health care professions. Patient Educ Couns. 2013; 93:18-26. www.researchgate.net/profile/RainerHaak/publication/233825938_A_European_consensus_on_learning_objectives_for_a_core_communication_curriculum_in_health_care_professions/links/5a09879baca272ed27a026e2/A-European-consensus-on-learning-objectives-for-a-core-communication-curriculum-in-health-care professions.pdf.
8. Lim RBT, Teng CWC, Azfar J, Bun D, Goh GJ, Lee JJM. An integrative approach to needs assessment and curriculum development of the first public health major in Singapore. 2020. Front. Public Health. 2020; 8 (182). doi: 10.3389/fpubh. 2020.00182
9. Chan TM, Jordan J, Clarke SO, Lawson L, Coates WC, Yarris LM, Santen SA, Gottlieb M. Beyond the CLAIM: a comprehensive needs assessment strategy for creating an advanced medical education research training program. AEM Educ Train. 2022;6:e10720. doi: 10.1002/aet2.10720
10. Allouch S, Ali RM, Al-Wattary N, Nomikos M, Marwan F. Abu-Hijleh MF. Tools for measuring curriculum integration in health professions' education: a systematic review. BMC Med Educ. 2024; 24: 635. https://doi.org/10.1186/s12909-024-05618
11. University of Papua New Guinea. School of Medicine and Health Sciences Course Handbook. 2024. Port Moresby. PNG
12. Rasenberg E, Brand G, van Weel-Baumgarten E. Integrating medical and practical skills in communication skills training: do students feel it supports them with transfer from classroom to practice? 2023.PECInnovation.2023;2(2023)100158. http://dx.doi.org/10.1016/j.pecinn.2023.100
13. Echeverri M, Chen AMH. Educational interventions for culturally competent healthcare: developing a protocol to conduct a systematic review of the rationale, content, teaching methods, and measures of effectiveness. 2016. J Best Pract Health Prof Divers. 2016; 9(1): 1160-1177.https://pmc.ncbi.nlm.nih.gov/articles/PMC8294596/pdf/nihms-1625713.pdf
14. Hauer J, Quill T. 2010. Educational needs assessment, development of learning objectives and choosing a teaching approach. J Palliat Med. 2010;14(4). https://doi.org/10.1089/jpm.2010.0232
15. Lepre B, Palermo C, Kylie J. Mansfield KJ, Beck EJ. Stakeholder engagement in competency framework development in health professions: a systematic review. Front. Med. 2021;8:759848. doi: 10.3389/fmed.2021.759848
16. Erjavec J. Stakeholders in curriculum development – case of supply chain and logistics programme. 7th International Conference on Higher Education Advances (HEAd'21).2021.doi:http://dx.doi.org/10.4995/HEAd21.2021.12875.
17. Belita E, Carter N, Bryant-Lukosius D. Stakeholder engagement in nursing curriculum development and renewal

initiatives: a review of the literature. Qual Adv Nurs Educ. 2020;6(1). doi: https://doi.org/10.17483/2368-6669.1200
18. Fąferek J, Cariou PL, Hege I, Mayer A, Morin L, Rodriguez-Molina D, Sousa-Pinto B, Kononowicz AA. Integrating virtual patients into undergraduate health professions curricula: a framework synthesis of stakeholders' opinions based on a systematic literature review. 2024. BMC Med Educ. 2024;24:727. https://doi.org/10.1186/s12909-024-05719
19. Denniston C, Molloy E, Nestel D, Woodward-Kron R, Keating JL. Learning outcomes for communication skills across the health professions: a systematic literature review and qualitative synthesis. BMJ Open. 2017;7:e014570. doi:10.1136/bmjopen-2016-014570
20. Nawagi F, Munabi IG, Vyt A. Using the modified Delphi technique to develop a framework for inter-professional education during international electives in health professions training institutions in Sub-Saharan Africa. Front Med. 2023. doi: 10.3389/fmed.2023.1225475
21. Li Y, Zheng D, Ma L, Luo Z, Wang X. Competency-based construction of a comprehensive curriculum system for undergraduate nursing majors in China: an in-depth interview and modified Delphi study. Ann Palliat Med. 2022;11(5):1786-1798. https://dx.doi.org/10.21037/apm-22
22. Engel KG, Pedersen K, Johansen MD, Shoennemann KR, Kjaer LB, Nayahangan LJ. Consensus on communication curricula content in Danish undergraduate medical education: a Delphi study. Med Teach. 2022;44(11):1221-1227. doi: 10.1080/0142159X.2022.2072280
23. Nasa P, Jain R, Juneja D. Delphi methodology in healthcare research: how to decide its appropriateness. World J Methodol.2021;11(4):116-129. https://dx.doi.org/10.5662/wjm.v11.i4.116.
24. Farrukh K. Enhancement of curriculum transparency in health professional education for students' progression and social accountability: empowering students. Health Professions Education and Research (HPER). 2023;1(5). https://doi.org/10.54844/hper.2023.046
25. Pitout H, Adams F, Casteleijn D, Henrietta S, du Toit J. Factors to consider in planning a tailored undergraduate inter-professional education and collaborative practice curriculum: a scoping review. S. Afr. J. Occup. Ther. 2022;52(1). https://doi.org/10.17159/23103833/2022/vol52n1a9
26. Kirwan M. Basic Communication Skills. In Gray C, Jenny Moffett J, eds. Handbook of Veterinary Communication Skills. 2009. https://download.ebookshelf.de/download/0000/5948/06/L-G-0000594806-0002364825.pdf
27. Hannah A, Millichamp JC, Kathryn M.S, Ayers MDS. A communication skills course for undergraduate dental students. J Dent Educ.2004;68(9).https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=fd8e3e5d078260658a258d2c5570a7c6b4e15061
28. Govindaraju P. Communication skills: an ignored component in dental education. J DentSciRes.2016;7(2).https://www.jdsr.co.in/vol7-issue1-new/1.pdf
29. World Health Organization. WHO Global Strategy on Human Resources for Health: Workforce 2030. Geneva: WHO 2016. https://iris.who.int/bitstream/handle/10665/250368/9789241511131-eng.pdf.
30. National Department of Health. National Human Resources for Health (HRH) Strategic Plan 2021-2030. n.d. Port Moresby: Papua New Guinea.

SHORT COMMUNICATION:

CONTEMPORARY NITI ROTARY INSTRUMENTATION AND THE INTEGRITY OF ROOT DENTIN: DOES CUTTING-EDGE SHAPING CREATE FUTURE FRACTURES? — A NARRATIVE REVIEW

[1]TONY FRANCIS, [2*]VIJAYENDRANATH S. NAYAK, [3]ROMA MASCARENHAS, [4]KARTHIK KANNAIYAN, [5]SAPTARSHI BHOWAL,

1. Department of Conservative Dentistry and Endodontics, Faculty of Dentistry, Manipal University College Malaysia, Melaka, Malaysia.
2. Department of Oral Medicine and Oral Radiology, Faculty of Dentistry, Manipal University College Malaysia, Melaka, Malaysia.
3. Department of Conservative Dentistry and Endodontics, Manipal College of Dental Sciences, Mangalore, Manipal Academy of Higher Education, Karnataka, India
4. Department of Prosthodontics, Faculty of Dentistry, Manipal University College Malaysia, Melaka, Malaysia.
5. Faculty of Dentistry, Manipal University College Malaysia, Melaka, Malaysia

*Correspondence author: drnayakomr@gmail.com

Submitted: July 2025. Accepted: October 2025

ABSTRACT

Nickel-titanium (NiTi) rotary instruments have transformed endodontics by enabling efficient, predictable root canal shaping. However, concerns persist about their impact on dentin integrity, particularly microcrack formation and long-term fracture risks. This narrative review evaluates evidence on contemporary NiTi systems—including thermally treated alloys and reciprocating files—and their effects on dentin structural integrity. While advanced designs reduce torsional stress, in vitro studies report conflicting outcomes on microcrack induction. Clinicians must balance shaping efficiency with preservation of dentin strength. Further in vivo studies are needed to correlate laboratory findings with clinical outcomes.

Keywords: Nickel-titanium, rotary instrumentation, dentin microcracks, root fracture, endodontics.

INTRODUCTION

Root canal preparation is a critical step in endodontic treatment, aiming to eliminate infected tissue while preserving dentin structure. The introduction of nickel-titanium (NiTi) rotary instruments in the 1990s marked a paradigm shift from stainless steel files, offering superior flexibility and resistance to torsional fracture [1]. Contemporary NiTi systems now incorporate thermally treated alloys (e.g., CM Wire, Gold

Wire) and adaptive motions (reciprocation, continuous rotation), promising enhanced safety and efficiency [2].

Despite these advancements, studies suggest that rotary instrumentation may induce microcracks in root dentin, potentially predisposing teeth to vertical root fractures (VRFs) [3].

This review examines:

- The evolution of NiTi systems and their biomechanical impact on dentin.
- Evidence linking instrumentation techniques to dentinal defects.
- Strategies to minimize structural compromise during shaping.

Evolution of NiTi Rotary Systems:

First- and Second-Generation Files

Early NiTi instruments (e.g., ProFile, LightSpeed) improved upon stainless steel files but had limitations in cyclic fatigue resistance and taper variability [4]. Second-generation systems like ProTaper Universal introduced progressive tapers, reducing canal transportation but increasing torsional stress [5].

Third-Generation Innovations:

Modern systems leverage metallurgical advancements:

Thermally treated alloys (e.g., HyFlex CM, EdgeSequel): Exhibit controlled memory and enhanced flexibility, reducing apical stress [6].

Reciprocating motion files (e.g., WaveOne Gold, Reciproc): Single-file systems that minimize cyclic fatigue via reverse cutting motions [7].

Offset design instruments (e.g., TRUShape): Asymmetric tapers preserve pericervical dentin [8].

Mechanisms of Dentin Damage During Instrumentation:

Microcrack Formation:

Rotary files generate frictional heat and shear stress, leading to microcracks, particularly in the apical third.[9] Adorno et al demonstrated that crack propagation correlates with file kinematics (rotation vs. reciprocation) and taper size [10].

Dentin Removal and Structural Weakening:

Excessive taper preparation (e.g., >6%) reduces radicular dentin thickness by up to 30%, compromising fracture resistance [11]. Bier et al [12] found that ProTaper F2 instruments caused deeper cracks than Mtwo files due to aggressive taper design.

Cyclic Fatigue and Torsional Stress

File fracture and dentin defects are linked to:

High RPM (>300): Increases screw-in forces [13].

Insufficient irrigation: Leads to dentin dehydration and brittleness [14].

Evidence on NiTi Systems and Dentin Integrity:

Studies Reporting Microcrack Induction

Liu et al compared hand files with rotary systems (ProTaper, K3) and found a 2.5× higher crack incidence with rotary use [15].

Bürklein et al [16] reported that reciprocating files (WaveOne) caused fewer cracks than continuous rotation systems (ProTaper).

Studies Refuting Significant Damage:

Versiani et al observed no difference in fracture resistance between teeth instrumented with Reciproc and hand files [17].

Pedullà et al [18] demonstrated that thermally treated files (HyFlex CM) produced fewer defects than conventional NiTi.

Factors Influencing Dentin Preservation

1. Instrumentation Technique:

Crown-down approach: Reduces apical stress by pre-flaring coronal dentin [19].

Single-file systems: Minimize repetitive dentin contact (e.g., XP-endo Shaper) [20].

2. Alloy Properties:

CM Wire: Lower elastic modulus reduces stress transmission [21].

Gold-treated files: Enhance fatigue resistance (e.g., Gold Wire by Dentsply) [22].

3. Operational Parameters:

Optimal torque (1–2 Ncm) and RPM (250–300) limit crack initiation [23].

Pecking motion (3–5 mm amplitude) prevents screw-in effects [24].

4. Irrigation and Lubrication:

Copious irrigant delivery together with chelating gel or liquid lubricants during rotary root canal preparation helps keep dentine hydrated and significantly reduces torque and apically directed forces on NiTi instruments, thereby decreasing the mechanical stresses at the file–dentin interface that are implicated in dentinal microcrack formation and root dentin defects [25].

Clinical Implications and Future Directions:

While in vitro data suggest that advanced NiTi systems reduce dentinal damage, clinical evidence remains sparse.

Recommendations include:

Selecting minimally invasive systems: TRUShape or HyFlex EDM for curved canals [26]. Adopting hybrid techniques: Combine rotary and hand files in fragile roots [27].

Long-term monitoring:

VRFs may manifest years post-treatment; CBCT follow-ups are advised [28]. Emerging technologies like AI-guided shaping and 3D-printed customized files could further optimize dentin preservation [29].

CONCLUSION

Contemporary NiTi systems offer unparalleled efficiency but require careful technique to avoid dentinal damage. Thermally treated and reciprocating files show promise in minimizing

microcracks, though clinicians must tailor instrumentation to root anatomy. Future research should prioritize in vivo studies correlating laboratory findings with clinical fracture rates.

REFERENCES

1. Walia HM, Brantley WA, Gerstein H. An initial investigation of the bending and torsional properties of Nitinol root canal files. J Endod. 1988;14(7):346-51.
2. Gavini G, Santos MD, Caldeira CL, Machado MEL, Freire LG, Iglecias EF, Peters OA, Candeiro GTM. Nickel-titanium instruments in endodontics: A concise review of the state of the art. Braz Oral Res. 2018;32(suppl 1):e67.
3. Adorno CG, Yoshioka T, Suda H. Crack initiation on the apical root surface caused by three different nickel-titanium rotary files at different working lengths. J Endod. 2011;37(4):522-5.
4. Peters OA. Current challenges and concepts in the preparation of root canal systems: a review. J Endod. 2004;30(8):559-67.
5. Ruddle CJ. The ProTaper technique. Endod Topics. 2005;10:187-90.
6. Alapati SB, Brantley WA, Svec TA, Powers JM, Nusstein JM, Daehn GS. SEM observations of nickel-titanium rotary endodontic instruments that fractured during clinical use. J Endod. 2005;31(1):40.
7. De-Deus G, Silva EJ, Vieira VT, Belladonna FG, Elias CN, Plotino G, Grande NM. Blue thermomechanical treatment optimizes fatigue resistance and flexibility of the Reciproc files. J Endod. 2017;43(3):462-6.
8. Lopes HP, Vieira MV, Elias CN, Gonçalves LS, Siqueira JF Jr, Moreira EJ, Vieira VT, Souza LC. Influence of the geometry of curved artificial canals on the fracture of rotary nickel-titanium instruments subjected to cyclic fatigue. J Endod. 2020;46(8):1102.
9. Liu R, Hou BX, Wesselink PR, Wu MK, Shemesh H. The incidence of root microcracks caused by 3 different single-file systems versus the ProTaper system. J Endod. 2013;39(8):1054-6.
10. Bürklein S, Tsotsis P, Schäfer E. Incidence of dentinal defects after root canal preparation: Reciprocating versus rotary instrumentation. J Endod. 2013;39(4):501.
11. Bier CA, Shemesh H, Tanomaru-Filho M, Wesselink PR, Wu MK. The ability of different nickel-titanium rotary instruments to induce dentinal damage during canal preparation. J Endod.2009;35(2):236-8.
12. De-Deus G, Belladonna FG, Souza EM, Silva EJ, Neves Ade A, Alves H, Lopes RT, Versiani MA. Micro-computed tomographic assessment on the effect of ProTaper Next and Twisted File Adaptive systems on dentinal cracks. J Endod. 2015;41(7):1116.
13. Pedullà E, Corsentino G, Ambu E, Rovai F, Campedelli F, Rapisarda S, La Rosa GR, Rapisarda E, Grandini S. Influence of continuous rotation or reciprocation of Optimum Torque Reverse motion on cyclic fatigue resistance of nickel-titanium instruments. Int Endod J. 2017;50(8):762-8.
14. Zuolo ML, De-Deus G, Belladonna FG, Silva EJ, Lopes RT, Souza EM, Versiani MA, Zaia AA. Micro-CT evaluation of dentinal microcracks after root canal preparation with TRUShape and Self-Adjusting File systems. J Endod. 2017;43(4):619-22.
15. Elnaghy AM, Elsaka SE. Evaluation of root canal transportation, centering ratio, and remaining dentin thickness associated with ProTaper Next instruments with and without glide path. J Endod. 2017;43(3):450-4.
16. Silva EJNL, Giraldes JFN, de Lima CO, Vieira VTL, Elias CN, Antunes HS. Influence of heat treatment on torsional resistance and surface roughness of nickel-titanium instruments. Int Endod J. 2019;52(11):1645-51.
17. Ha JH, Kim SK, Cohenca N, Kim HC. Effect of R-phase heat treatment on torsional

resistance and cyclic fatigue fracture. J Endod. 2013;39(3):389-93.

18. Plotino G, Grande NM, Testarelli L, Gambarini G. Cyclic fatigue of Reciproc and WaveOne reciprocating instruments. Int Endod J. 2012;45(7):614-8.
19. Arias A, Perez-Higueras JJ, de la Macorra JC. Differences in cyclic fatigue resistance between ProTaper Next and ProTaper Universal instruments at different levels. J Endod. 2014;40(9):1477-81.
20. Hieawy A, Haapasalo M, Zhou H, Wang ZJ, Shen Y. Phase transformation behavior and resistance to bending and cyclic fatigue of ProTaper Gold and ProTaper Universal instruments. J Endod. 2015;41(7):1134-8.
21. Pirani C, Iacono F, Generali L, Sassatelli P, Nucci C, Lusvarghi L, Gandolfi MG, Prati C. HyFlex EDM: superficial features, metallurgical analysis and fatigue resistance of innovative electro discharge machined NiTi rotary instruments. Int Endod J. 2016;49(5):483-93.
22. Uygun AD, Kol E, Topcu MK, Seckin F, Ersoy I, Tanriver M. Variations in cyclic fatigue resistance among ProTaper Gold, ProTaper Next and ProTaper Universal instruments at different levels. Int Endod J. 2016;49(5):494-9.
23. Jamleh A, Komabayashi T, Ebihara A, Nassar M, Watanabe S, Yoshioka T, Miyara K, Suda H. Root surface strain during canal shaping and its influence on apical microcrack development: a preliminary investigation. Int Endod J. 2015;48(12):1103-11.
24. Ozyurek T, Yılmaz K, Uslu G. Shaping ability of Reciproc, WaveOne GOLD, and HyFlex EDM single-file systems in simulated S-shaped canals. J Endod. 2017;43(5):805-9.
25. Peters OA, Boessler C, Zehnder M. Effect of liquid and paste-type lubricants on torque values during simulated rotary root canal instrumentation. Int Endod J. 2005 Apr;38(4):223-9.
26. Topcuoglu HS, Topcuoglu G. Cyclic fatigue resistance of Reciproc Blue and Reciproc files in an S-shaped canal. J Endod. 2017;43(10):1679-82.
27. Kiefner P, Ban M, De-Deus G. Is the reciprocating movement per se able to improve the cyclic fatigue resistance of instruments? Int Endod J2014;47(5):430-6.
28. Schneider SW. A comparison of canal preparations in straight and curved root canals. Oral Surg Oral Med Oral Pathol. 1971;32(2):271-5.
29. Zanza A, Seracchiani M, Reda R, Miccoli G, Testarelli L, Di Nardo D. Metallurgical tests in endodontics: a narrative review. Bioengineering 2022; 9 (1): 30.

SHORT COMMUNICATION:

BEYOND THE LAST BREATH: A MICRO-TOOLKIT FOR GRIEF SUPPORT IN MYANMAR FAMILIES

[1]SHOON MYA AYE and [2,3]SHYH POH TEO*

1. Karuna Compassionate Care Center, Yangon, Myanmar
2. Geriatrics and Palliative Medicine, RIPAS Hospital, Brunei Darussalam
3. PAPRSB Institute of Health Sciences, Universiti Brunei Darussalam, Brunei Darussalam

*Corresponding Author: shyhpoh.teo@ubd.edu.bn

Submitted; October 2025. Accepted: November 2025

INTRODUCTION:

Grief is a universal human experience, yet the experience is profoundly contextual. Grief, the behavioral and emotional response to bereavement, is different for everyone. Different cultures also have their own unique rituals, traditions and practices to respond to the loss. These practices offer mourners pathways to express emotions, honor memories of the deceased, and reintegrate back into daily life [1]. In Myanmar, bereavement is deeply intertwined with Buddhist teachings; values of impermanence (anicca), compassion or wish for people to be free from suffering (karuna) and community solidarity. Monasteries, family homes, and community gatherings provide spaces for mourning, while practices such as offering alms or chanting sutras are intended to ease the transition for the deceased and comfort the bereaved [2]. However, there is little systematic development of compassionate bereavement support tools that align with psychological insights on grief and adaptations to local cultural practices [3].

The grief theory describes the active processes through which individuals cope, starting from accepting the reality of loss, processing pain, adjusting to a new environment, and finding enduring connections [4]. While cultural bereavement practices guide people through this process, urbanization, migration and limited formal bereavement services make it difficult for grieving families to maintain these traditional practices [5]. Approximately 10% of grief is complicated, where there is difficulty accepting or adjusting to the loss, or ongoing traumatic distress beyond six months [6]. As there is significant variability in the provision of formal bereavement support [7], we propose a set of culturally resonant micro-practices for grief support among Myanmar families. These are

intentionally small, accessible acts of compassion that can be used in homes, monasteries, or healthcare settings to support bereaved families.

Proposed Micro-Practices for Bereavement Support:

Silent Listening Ritual:

Sit together for several minutes of intentional silence, without offering advice, distraction or judgement. Silence is a form of emotional expression, without pressure on a person to articulate their grief. Presence is a compassionate act to show they are not alone. "*It's okay to feel sad. Grief is not weakness; it is love that has lost its voice*".

This can be done during home visits, memorial gatherings, or hospital follow-ups to normalize grief without forcing conversations.

Dhamma Candle Offering Ritual:

Find a quiet space at home. Light a candle or oil lamp in memory. While watching the flame, offer a moment of silence, make a wish in your heart and send your love and blessings to them. "*As these light shines, may your next journey be peaceful. Your love continues to shine brightly, even in our darkest hours*".

Families may incorporate this ritual daily, during anniversaries or religious observances, as a way to remember their loved ones and send blessings for the onward journey.

Breathing with compassion (karuna breathing):

Sit and breathe gently. With each inhale, accept your grief and know that this sadness is a natural part of your love. With each exhale, send compassion (karuna) to your own grieving heart and loving kindness to your loved one. "*May I be gentle with my grief. May my loved one be free from suffering and find peace*".

Compassion-focused breathing helps to regulate distress and build self-soothing capacities [8]. This can be carried out individually during moments of acute grief, or in small groups at home, monasteries, or community gatherings.

Compassionate Legacy Letter Writing:

Write a letter to the one you lost. Use this space to express gratitude, unspoken thoughts or blessings. The letter can be kept privately, placed on a family altar, or ritual burned to symbolize release and the sharing of merit. This act of expression helps clear your heart, allows you to honor their actions (kamma) and find peace in their memory.

While this practice is not a Myanmar tradition, it is an emerging practice in urban communities, where individuals write letters to loved ones before death, especially in palliative care settings. This is increasingly used by clinicians and spiritual caregivers to help patients articulate their values, regrets, hopes, and blessings. Expressive writing also facilitates emotional processing and meaning making, providing a safe outlet for complex emotions [9].

This provides a private, flexible space for grief expression, is useful for younger family members, or those less comfortable with rituals, and is currently being explored as a tool for dignity therapy and emotional closure.

Compassionate Check-Ins:

Healthcare providers, family members, neighbors, or community leaders send a note, short phone call, or text message several weeks after the funeral to remind families that they are not forgotten. "*We are thinking of you. You are not alone*".

While condolence notes and community solidarity are common in Myanmar, extending these gestures beyond the immediate funeral period sustains compassionate presence and remind families that they are not forgotten. Hospitals, clinics and monasteries can integrate this as a simple follow-up practice for bereaved families [10].

DISCUSSION:

These micro-practices are deliberate actions to empower families to take ownership of the grief process. They can be integrated into daily routines and do not require specialized training. Professional bereavement support and counselling may be inaccessible or unavailable and should not be limited to clinical settings. While these actions are rooted in Myanmar traditions, they have the potential for adaptation elsewhere. For example, letter writing may resonate in Western contexts, while silent presence may be universal.

These practices also illustrate compassion as an embodied, relational process. Compassion here is not a grand intervention but a quiet presence, such as lighting a candle, writing a note, or breathing with awareness. Such acts reaffirm the bereaved person's dignity and humanity, reminding them they are seen and supported.

This proposed approach is reflective, and practice-based rather than empirical; and is not aimed to be prescriptive, but to inspire culturally responsive grief care. The micro-practices require further evaluation regarding acceptability, cultural resonance, and perceived impact; as well as an exploration of integrating these practices into palliative care follow-up.

CONCLUSION:

Bereavement is never an easy journey and often carried quietly with limited formal support by Myanmar families.

The micro-practices described from silent listening, candle rituals, compassionate breathing, letter writing, and compassionate check-ins are rooted in Buddhist values, intentionally small in scale, accessible and may have profound impact on families. It is hoped that these micro-practices will empower families and communities to accompany each other in grief, transform silent suffering into shared healing, and provide compassion beyond the last breath.

REFERENCE:

1. Cacciatore J, Thieleman K, Fretts R, Jackson LB. What is good grief support? Exploring the actors and actions in social support after traumatic grief. PLoS One 2021;16(5):e0252324
2. Wada K, Park J. Integrating Buddhist psychology into grief counseling. Death Stud 2009;33(7):657-683.
3. Kohler M, Thaqi Q, Kuske S, Schwalbach T, Riguzzi M, Naef R. Evidence adaptation and tailored implementation of family bereavement support in specialized palliative care: a multi-method study. Int J Nurs Stud Adv 2025; 8:100305.
4. Stroebe M, Schut H, Boerner K. Continuing bonds in adaptation to bereavement: toward theoretical integration. Clin Psychol Rev 2010;20(2):259-268.
5. Aoun SM, Breen LJ, White I, Rumbold B, Kellehear A. What sources of bereavement support are perceived helpful by bereaved people and why? Empirical evidence for the compassionate community's approach. Palliat Med 2018;32(8):1378-1388.
6. Zisook S, Shear K. Grief and bereavement: what psychiatrists need to know. World Psychiatry 2009;8(2):67-74.
7. Efstathiou N, Walker W, Metcalfe A, Vanderspank-Wright B. The state of bereavement support in adult intensive care: a systematic review and narrative synthesis. Journal Crit Care 2019; 50:177-187.
8. McAdam JL, Erikson A. Self-care in the bereavement process. Crit Care Nurs Clin 2020;32(3):421-437.
9. Pennebaker JW. Expressive writing in psychological science. Perspect Psychol Sci 2017;13(2):226-229.
10. Breen LJ. Harnessing social support for bereavement now and beyond the COVID-19 pandemic. Palliat Care Soc Pract 2021; 15:2632352420988009.

INSTRUCTIONS TO AUTHORS:

AIMS AND SCOPE:

Pacific Journal of Medical Sciences is a peer-reviewed, multidisciplinary journal published by the School of Medicine and Health Sciences (SMHS), University of Papua New Guinea (UPNG).

The aim of The Pacific Journal of Medical Sciences is to provide the forum for researchers, medical practitioners and other professionals to record, publish, and share ideas and research findings that serve to enhance the understanding of the aetiology, symptoms, diagnosis, prevention, control and management of human disease conditions worldwide.

The Pacific Journal of Medical Sciences publishes original scientific research reports, case reports, short communications, letters to the editor and reviews, representing new and significant findings in all areas of medical, biomedical and health sciences (including epidemiology, public and environmental health). Book reviews, scientific news and conference proceedings are published on special request.

EDITORIAL POLICIES:

The Pacific Journal of Medical Sciences (Pac J Med Sci) editorial policies require that: All manuscripts (papers) accepted for publication must reflect novelty and originality of quality research and be of interest to a multidisciplinary audience. All papers submitted for publication are bench reviewed. If the bench review report of a paper is positive, the paper is then sent for peer-review by three anonymous reviewers. If two of the three reports are positive, then the editor-in-chief or a member of the editorial board will review the paper further. If the report is positive, the paper will be sent to the corresponding author for corrections to be made. The corrected paper will be sent to one or two of the reviewers for rechecking. After rechecking, the paper will then be sent for technical review. The report of the technical reviewer will determine if the paper will be accepted for publication.

The editorial board may request review articles, commentaries or short reviews on contemporary medical or biomedical issues that the board considered important to the advancement of the aims of the journal.

Original research papers should be both complete and concise; they should essentially offer conclusive results, but they should not exceed 7,500 words, including abstract, tables, figures and references.

Short communications and reports should not exceed 3500 words, including abstract, tables, figures and references. Review articles should not exceed 6,000 words, including tables, figures and references. Letter to the Editor should be brief and to the point.

On preliminary editing, all manuscripts that fail to meet the basic requirements indicated above and those that contain significant and obvious typographical errors are returned without further processing.

Manuscripts submitted will be reviewed and considered for publication only if they have not been published, simultaneously submitted or already accepted for publication in another journal. The author responsible for correspondence must show evidence of approval of all co-authors when submitting a paper for publication.

All relevant ethical approval for research involving human and animal subjects must conform to the provisions of the Declaration of Helsinki in 1995 (as revised in Edinburgh 2000).

Only research methods that comply with internationally accepted principles of humane animal experimentation are accepted for publication in The Pacific Journal of

Medical Sciences. The decision to accept, revise or reject any manuscript for publication in The Pacific Journal of Medical Sciences is the responsibility of the editor-in-chief; this is done after reviewing the reports and comments from the reviewers, and technical reviewers in consultation with members of the editorial board.

Disclaimer:

All statements and opinions expressed in any of the manuscripts published in The Pacific Journal of Medical Sciences are of the authors and co-authors, and not necessarily of the editors or members of the editorial board.

The editor-in-chief and members of the editorial board of The Pacific Journal of Medical Sciences disclaim any responsibility or liability for such material and do not guarantee or endorse any products or services mentioned in the articles, nor guarantee any claims made by the authors of the articles.

SUBMISSION OF MANUSCRIPT (PAPER):

Manuscript should be written in clear and concise English and be intelligible to those that are not specialists in the particular scientific area. Manuscript that does not satisfy these requirements but is acceptable for publication in the Pacific Journal of Medical Sciences because of its essential scientific content will be returned to the authors for extensive and appropriate revision, as recommended by the reviewers and editors.

A covering letter to clarify the following should accompany any manuscript submitted for publication in the Pacific Journal of Medical Sciences: (a) That the scientific data contained in the manuscript has not been published or submitted for publication in any other journal; (b) That ethical clearance and permission for the research had been obtained from the appropriate committee(s) in the institution(s) where the work was carried out; (c) That all the authors have read and approved the content of the manuscript; (d) The name, address and email contact of the author responsible for correspondence and for communicating with others about revisions and final approval of the galley proof of the manuscript; (e) Statements quantifying the contribution of each author to the manuscript (*this is a requirement in the latest guidelines of the International Committee of Medical Journal Editors*).

Only electronic copy of the manuscript sent as e-mail attachment should be submitted using the approved format indicated in the appropriate sections of this document.

Manuscript should be sent by email to any of the following: managingeditorpjms1625@gmail.com; pacjmedsci1625@gmail.com; pacjmedsci@gmail.com.

PREPARATION OF MANUSCRIPT / PAPER:

Manuscripts should be prepared **on A 4 paper**, using double-spacing. Pages are to be numbered consecutively in the bottom right-hand corner. Manuscript should include the following sections: Title page, abstract, keywords, main text, acknowledgements, references, tables and figures.

Style: The Pacific Journal of Medical Sciences uses both UK and US spelling. Only one or the other should be used throughout a manuscript. SI units should be used for all measurements. Use abbreviations or acronyms to avoid repetition of long technical terms: Indicate the abbreviation in parentheses when the word is used in full for the first time. Use the approved generic names of chemical substances and drugs. Do not use trade names or brand names of chemicals and drugs.

Title page: The following should be on the title page: (a) Title of the manuscript – it should be concise and informative; (b) Short running title of not more than 40 characters (optional); (c) Name of each author (first name, middle initial and last name); (d) Name and address of institution(s) in which the work was carried out; (e) Name,

postal address and email contact of the author responsible for correspondence; Source(s) of research or other types of support for the research project, if any,

Abstract and key words:

The abstract should not be more than 300 words. The following should be clearly stated in the abstract: the purpose of the study, basic procedures, main findings (specific results and statistical significance, if any), and principal conclusions. Abbreviations and references should not be included in the abstract. Not more than 8 key words should be put below the abstract. Key words are used to assist indexers in cross-indexing published articles and may be published with the abstract. Medical Subject Headings (MeSH) list of the Index Medicus should be used for selecting key words (www.nlm.nih.gov/mesh/meshhome.html)

Main Text:

Text of an original manuscript / paper should be separated into the standard IMRAD format as follows: Introduction, Materials and Methods (Methodology), Results, Discussion. Sections on Acknowledgements and References should be included.

Introduction: This section should: (a) summarize relevant previous work, using appropriate references, without any extensive review of the subject; (b) clearly state the purpose of the study and summarize the rational for the study or observation; (c) avoid giving any data on the work being reported.

Materials and Methods (Methodology): This section should: (a) clearly indicate either the sampling procedure or observational subjects; (b) give appropriate references for established techniques and procedures; (c) new techniques and procedures and extensive modifications of existing ones should be presented in sufficient details so that other researchers can easily reproduce and evaluate them; (d) indicate appropriate quality control procedures used for laboratory methods and techniques; (e) indicate ethical procedures if either human subjects were involved [if informed consent was obtained from each subject] or if appropriate guide line for using laboratory animals were followed [see editorial policies above]; (f) indicate statistical methods used, if any.

Results: Data obtained should be presented in logical sequence in the text, tables and figures should be adequately explained to facilitate their interpretation. Avoid duplicating the results by repeating in the text all the data presented in the tables and figures. Import to include the values on all figures/ graphs. **Cutting and pasting of figures or graphs is not acceptable**. The text in the results section should only emphasize or summarize the important data.

Discussion: Major findings should be highlighted before the minor findings. All findings should be related to other relevant studies, if any, using appropriate references. Indicate the implications of the findings and their significance or limitations, including implications for future research. When warranted, propose new hypotheses with appropriate data, but be sure to clearly label them as such.

Conclusions: should be linked with the goals of the study and be clearly supported by evidence / data. Include recommendations, if applicable.

Acknowledgements:

The following should be acknowledged: Research or other financial grants; Material support, Contributions of Institutions, Colleagues, and other relevant participants.

References:

The Pacific Journal of Medical Sciences uses the Vancouver system of referencing. The references should be numbered, using Arabic numerals in square brackets [], in the order in which they are first used in the text, tables, figures, and legends.

In the reference section, list the references in the order of appearance in the text, tables, figures and legends.

Abstracts, unpublished data, oral communications, and personal communications should not be included in the reference section.

All references should be verified against the original documents.

In the reference section, the names of all authors should be included. **Avoid using "*et al.*"** in the reference section. Names of journals should be abbreviated, using the approved style indicated in Index Medicus/PubMed.

References should be listed according to the examples given below:

Journal articles:

Gima E and Vince JD. The Brixia Chest X-Ray Severity Score in Adult Patients with Symptomatic COVID-19 Infection: A Useful Guide to Management: Pac J Med Sci. Vol. 23, No 2, January 2023: 16 – 24.

Book:

Berdanier, C.D., & Berdanier, L.A. (2021). Advanced Nutrition: Macronutrients, Micronutrients, and Metabolism (3rd ed.). CRC Press.

Chapter in a Book:

Delange F, Dunn JT. Iodine deficiency. In: The Thyroid. A Fundamental of Clinical Text. L E Braverman and R T Utiger eds. Lippincott, Williams & Wilkins publ. Philadelphia: 2005. pp 264 – 288.

Published proceedings paper:

Kruse JD. Basic principles of zinc metabolism. *In*: Kruse-Jarres JD, Scholmerich J, editors. Zinc and diseases of the digestive tract. Proceedings of an International workshop, Australia, 2019: 3 – 15.

Tables:

Tables should be numbered sequentially in Arabic numerals, typed single-space and included within the appropriate section in the text of the manuscript. Vertical lines can be used to separate columns. Each table should be self-contained with a comprehensive but concise legend/heading; column headings should be brief, with units in parenthesis. All non-standard abbreviations used in tables should be explained in footnotes, using the following symbols in this sequence: *, §, ¶, #, $.

Illustrations:

Graphs, line drawings, bar charts, maps, etc., should be labelled as 'figures' and numbered consecutively, using Arabic numerals. All figures should be drawn using computer graphics, **do not copy and paste figures**. Legends should be brief but understandable without referring to the text. Photographs should be black and white or colored, sharp, glossy, black and white prints. Photographs can contain scale bars, but not magnifications. *Figures, reproduced from another source, should be clearly indicated and appropriate references and written permission from the copyright holder must be submitted with the manuscript.*

Electronic copy of manuscripts (e-mail):

When a manuscript is accepted for publication, the corresponding author will be required to send an electronic copy of the corrected or modified manuscript by email. **All email attachments should be scanned with anti-virus software before sending**. **Automatic software for referencing, footnotes, headers, footers, etc., should NOT be used during formatting**.

All manuscripts should be formatted using MS WORD on A 4 size paper.

GALLEY PROOFS:

Galley proof will be sent by email to the correspondent author. Only minor corrections should be made, no major

alterations to the manuscript will be accepted. Galley proof should be returned within 7 working days from the date of receipt. If any major modification is made, the manuscript will be rejected at this stage. Correspondent author should correct all printing errors and ensure that the typesetting is accurate in the galley proof. Note that the correspondent author, not the editorial board of the Pacific Journal of Medical Sciences will be responsible for any such errors, should they occur in the published paper.

REPRINTS:

Reprints will not be sent to authors, because the PJMS is an on-line journal. The Web-link will be sent by email to the corresponding author. The full copy of the journal and individual papers in the journal will be available for downloading from the website. Authors are free to download and print the journal at their own expense.

COPYRIGHT:

Manuscripts accepted for publication in The Pacific Journal of Medical Sciences become the property of the journal. Therefore, all authors **are** requested to indicate that the copyright is transferred to the journal. The copyright gives the journal the exclusive rights to reproduce, translate and distribute the article only for academic purposes.

CHECKLIST FOR AUTHORS:

- Running title included (Can be omitted if not applicable).
- Abstract prepared according to instructions and included key words.
- Manuscript typed double-space on A 4 with normal margin.
- References cited in square brackets in text and listed according to approved style for this journal, Uniform spelling throughout the text.
- Tables and Figs located in the appropriate section within the text of the manuscript.
- Covering letter written as required by this journal;
- E-mail address of corresponding author indicated.