

Indexations
EMR Index Medicus
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Pakmedinet

p-ISSN 1819-270X
e-ISSN 2073-1183
IP-034
Pak J Physiol

PAKISTAN PHYSIOLOGICAL SOCIETY



PAKISTAN JOURNAL of Physiology

VOLUME 22 NUMBER 1 (JAN–MAR 2026)

BRIG (RETD) PROF DR SAADAT ALI KHAN

SI (M)



It is a difficult task to write an obituary for a person with whom one has spent a substantial part of life! I have known Brig Saadat Ali Khan since 1978 and remained in active contact with him till his last breath. He was born on 1 May 1948 and passed through a hard childhood during migration from India to newly created country, Pakistan. He faced a rugged time in early age in adverse circumstances but kept his dreams high. Later in 1975, he graduated his MBBS from Dow Medical College (now Dow Medical University), Karachi. He was a self-made person and used to teach (tuition) to FSc and Matric students in the evening hours to make his living. Soon after his graduation, he served as a Demonstrator in Physiology at DMC Karachi for about a year and then joined Pakistan Army in January 1976 and was posted as General Duty Medical Officer in Sialkot. At that time, Army Medical College got established and he was appointed as a Demonstrator in Physiology in April 1977. Thus, he had a pride to be a founding teacher of this great Institute!

He was selected to do M.Phil at Qaid-i-Azam University, Islamabad and qualified in 1980. He was promoted to Assistant and Associate Professor of Physiology and chaired the Department at Army Medical College up to 1993. He was then posted to Armed Forces Medical College (now Armed Forces Postgraduate Medical Institute), Rawalpindi in 1993 and served there till his retirement in 2005. In the meanwhile, as a Professor of Physiology FCPS in Physiology by the CPSP was launched in the country and Army Medical College, Rawalpindi was the pioneer institution to impart training in 1996. Brig Saadat joined FCPS-II training program and qualified FCPS in 1999 as one of the founding faculty member to accomplish this honour. He qualified MSc in Pain Medicine in 2015.

Upon retirement from Pakistan Army, he served as Professor and Head of Physiology Department at Foundation University Medical College Rawalpindi from 2005 to 2015. He served as Professor and Chairman of Physiology Department at Rai Medical College, Sargodha, Shahida Islam Medical and Dental College, Lodhran, and Multan Medical & Dental College, Multan from 2018 to 2024.

He was an immaculate teacher and very popular among the students. He had a vast experience of teaching both to undergraduate and postgraduate students and for the inter-professionals like FCPS, M.Phil, MBBS, BDS, Nursing and Allied Health Sciences at CPSP, PIMS, AFID, AIU, and at Tribhuvan University, Nepal. He conducted MBBS, BDS, M.Phil, and FCPS examinations at several centres in the country.

Neurophysiology was his area of interest in Academia and he researched in High Altitude Medicine and in the fields of sleep and Pineal Gland. He was part of the research expeditions at High Altitude in Kaghan Valley, Siachen, Chitral and Misqar. He has 30 publications in elegant journals to his credit. He has supervised researches leading to M.Phil and FCPS to 10 residents. He is a co-author of the books on 'Anaesthesia and Patient Care', 'Man, Mountain and Medicine', and 'Laboratory Manual in Physiology'. He has participated and conducted around one hundred workshops, symposia, seminars in Pedagogy and Andragogy. He remained an active member of several research organizations and a reviewer to many medical journals.

Brig Saadat was a founding member and treasurer of Pakistan Physiological Society (PPS) and South Asian Association of Physiologists (SAAP), and actively participated in the biannual conferences of the Societies in various cities of Pakistan, Bangladesh, Sri Lanka, Nepal, and India. His contributions for Pakistan Journal of Physiology (PJP), College Magazine of Army Medical College (AMCOL), and Foundation University Medical College (JFUMC) since their inception are applaudable. Brig Saadat was a unique combination of science and humanities. He was a writer, a poet, an author, a reader, an editor and advisor, and a reviewer in literary sciences. He had a good sense of humour and wit. He had a social circle of religious, literary, scientists and educational reformers around him. He was a noble, humble and a kind-hearted man. He substantially contributed to his sisters and the needy people in all the ways he could. He was a role model for his students and family. He was a classic example of an eastern man and took utmost care of the Islamic and eastern values.

He was a loving husband and a father and had a thundering shock of his life when his young son suddenly expired. He was a model of 'sabr' and his strong faith in God Almighty made it possible to absorb and not to reveal his sadness but to say *Al-Humdu Lillah*. That's what many of us would not possibly be able to do in grief. Brig Saadat has prostrated due to Congestive Cardiac Failure and during the last couple of years of his life was repeatedly hospitalized at CMH, Rawalpindi and at Armed Forces Institute of Cardiology (AFIC) Rawalpindi. He breathed his last at CCU, AFIC, Rawalpindi on 29 January, 2026. He left behind his learned wife, a daughter (Pathologist) and a charming grand daughter. His daughter is married to a Dental Surgeon, who is son of Lt Gen Mushtaq Ahmad Baig, Shaheed.

May Almighty Allah bless his soul with solace and in *Jannah*, Aameen. Someone said and I quote, 'It is not the length of life, but the depth of the life' and my friend was a real deep man!

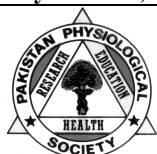
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Pakistan Journal of Physiology

Pak J Physiol 2026;22(1) pISSN 1819-270X, eISSN 2073-1183, IP/0034 Volume 22, Number 1 (Jan–Mar 2026)



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Page size= 8.5×11.0 inch, 4 colour and B/W, matt paper 90 gm/m², Cover= art card 250 gm/m², 4 colour printing, laminated, side stapled and glue binding. All margins= 1.0 inch, printed area 6.5×9 inch, 2 columns of 3 inch and 0.5 inch between columns.

Printed at Pictorial Printers (Pvt) Ltd., Aabpara, Islamabad, Pakistan



EDITORIAL

MEDICAL IMPORTANCE OF HUMAN MICROBIOTA

Tehseen Iqbal

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Microorganisms are always considered as pathogens but it is now estimated that less than 1% of known microbial species are pathogenic to humans. The most microbes either coexist 'peacefully' with humans and they are good microbes. Human microbiota inhabits the body, living on the skin, in the mouth, gut, nose, and genital tract. Although the importance of gut microbiota for digestion of foodstuffs is widely recognized, additional roles for the body's microbes in nutrition, immunity, and other functions is being recognized now. Human microbiota regulates brain, liver, and lung health via bidirectional pathways like the microbiota-gut-brain axis, influencing mood, cognitive function, and systemic inflammation. Modern medicine is increasingly targeting the microbiome to treat diseases by using probiotics, and prebiotics to restore balance, and by using faecal microbiota transplantation.

Keywords: Faecal microbiota transplantation, Human microbiota, Prebiotics, Probiotics

Pak J Physiol 2026;22(1):1–2, DOI: <https://doi.org/10.69656/pjp.v22i1.1945>

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We used to think about the microbes as 'bad' for humans. This was because of the popular 'germ theory' of disease which states that microorganisms can invade living hosts and cause a disease. Robert Koch made the discoveries that led Louis Pasteur to describe how small organisms called germs could invade the body and cause disease. Germ theory replaced the 'miasma theory' (bad air) during the late nineteenth century. This resulted in development of stringent sanitation measures and development of very effective vaccines and antibiotics. Microorganisms are always considered as pathogens but it is now estimated that less than 1% of known microbial species are pathogenic to humans. The most microbes either coexist peacefully with humans or are essential for life on Earth.¹ Now we recognize that there are 'good microbes', or commensal microbes also, that provide required help to the human and similarly would provide benefit to the environmental source.²

Although the first scientific evidence that microorganisms are part of the normal human system emerged in the mid-1880s, the concept of the human microbiome, and thus the intensive study of it, was developed primarily in the first decade of the 21st century. The Human Microbiome Project (HMP) was started in 2007 and in the first three years of the project, scientists discovered nearly 200 different bacterial member species of the human microbiota.³ Now, it is known that the human body is home to trillions of microorganisms, including bacteria, viruses, and fungi. In a healthy adult, this is called microbiota which collectively has a biomass equal to the human brain.⁴ The human microbiota consists of approximately 100 trillion microorganisms that is about three times more than the human cells. Human microbiota weighs around 1.1 Kg and occupies 1.4 litres of space. Their highest density is found in the intestines. These microorganisms interact with the host and profoundly influence physiological homeostasis and immune mechanisms.⁵

Human microbiota inhabits the body, living on the skin, in the mouth, gut, nose and genital tract. The gastrointestinal tract normally contains a complex, dynamic population of 400 to 1,000 species of microorganisms that outnumber human cells. Communities of microorganisms that inhabit the body can cause diseases, but most of the times they live in harmony with their human hosts and provide vital functions that are essential for survival of their hosts. Although the importance of gut microbiota for digestion of foodstuffs is widely recognized, additional roles for the body's microbes in nutrition, immunity, and other functions are just beginning to be appreciated and this represents an intensive area of biomedical research. In healthy humans, most gut microbes are strictly anaerobic and about 90% belong to the phyla *Bacteroidetes* and *Firmicutes*, although there are many different types of bacteria in each of these phyla.⁶

Some scientists consider human microbiota as a 'virtual organ' because it performs essential functions that the human genome cannot perform. Human microbiota synthesizes essential vitamins, e.g., Vitamin K, B12, folate, biotin. They break down indigestible fibres into short-chain fatty acids (SCFAs) like butyrate which is an energy source for colonic cells. Human microbiota regulates lipid and glucose metabolism. Human microbiota trains the immune system to distinguish between harmless and pathogenic microbes. It regulates inflammatory responses, and supports the integrity of mucosal barriers.

Commensal microbes prevent the overgrowth of pathogens by competing for nutrients and space and by producing antimicrobial substances called bacteriocins. Interestingly, human microbiota regulates brain, liver, and lung health via bidirectional pathways like the microbiota-gut-brain axis, influencing mood, cognitive function, and systemic inflammation.⁷

Although the gastrointestinal tract is

essentially sterile at birth, microorganisms living in symbiosis with one another and with the host rapidly colonize the gut after birth. The composition of the microbiota in infants is influenced by multiple factors, including the birth modality (Caesarean section versus vaginal delivery), diet (breast milk versus formula feeding), and the use of antibiotics. With the cessation of breastfeeding and the start of a more diverse diet, there is rapid diversification of gut microbiota. The concentration of these microbes increases steadily along the gastrointestinal tract, with relatively small numbers in stomach and extremely large numbers in colon.⁶

In patients with inflammatory bowel disease (IBD), there is a decrease in the number of bacteria from the phylum *Firmicutes*. Various environmental factors often trigger dysbiosis, so broad-spectrum antibiotics used to treat infections significantly impact the microbiota. Dysbiosis is of three types: Loss of beneficial bacteria, Overgrowth of potentially pathogenic bacteria, Loss of overall bacterial diversity.

The majority of doctors are not aware of the rapidly developing science of microbiome and they are not also trained to give evidence based nutritional advice. Western-style diet is associated with gut dysbiosis and inflammation, which in extreme cases, may result in a leaky gut and translocation of gut-derived bacteria that promote liver inflammation and non-alcoholic steatohepatitis (NASH).⁵ A diet that is high in plant-based carbohydrates, fats, grains, fish and naturally fermented foods is the healthiest diets around the world. Certain lifestyle changes and novel approaches including Faecal Microbiota Transplantation (FMT) and nutritional supplementation with probiotics, prebiotics, and symbiotics have offered solutions for dysbiosis management and paved the way towards restoring a healthy microbiome, with only minimal long-term unfavourable effects.⁹

Modern medicine is increasingly targeting the microbiome to treat diseases by the use of probiotics (live beneficial microbes) and prebiotics (fibre that feeds them) to restore balance; by using faecal microbiota transplantation which is now an established treatment for recurrent *C. difficile* and it is an investigational therapy for IBD and metabolic disorders. Developing personalized nutrition and ‘metabolism-based editing’ is to strengthen host functions that control the microbial environment.

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Received: 25 Mar 2026

Reviewed: 26 Mar 2026

Accepted: 26 Mar 2026

The author approved the draft and is accountable in ensuring that questions related to accuracy or integrity of the work are duly investigated and resolved.

Conflict of Interest: None

Funding: None



ORIGINAL ARTICLE

SERUM CYSTATIN C AS A BIOMARKER OF ACUTE KIDNEY INJURY IN PRE-ECLAMPTIC PATIENTS

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Background: Pre-eclampsia is a common multisystem disorder of pregnancy especially kidney damage. Cystatin C is a reliable marker to be used for kidney function. Objective of this study was to evaluate diagnostic accuracy of cystatin C in comparison of serum creatinine for the early diagnosis of acute kidney injury in patients of pre-eclampsia. **Methods:** This case control study was conducted in Department of Chemical Pathology of a tertiary care hospital in Rawalpindi from Jun 2024 to Apr 2025. A sample size of 70 (35 patients, 35 controls) with 95% confidence interval was calculated with 1:1 case to control ratio using online WHO calculator. Simple consecutive sampling was used. Patients diagnosed with pre-eclampsia with >20 weeks of gestation were included according to the American Obstetric and Gynaecologists guidelines. Controls were healthy pregnant mothers of the same gestational duration. Five mL of blood was collected from each patient for estimation of random blood glucose, serum creatinine, uric acid, and cystatin C. Statistical analysis was performed using Pearson's correlation, *t*-test and ROC curve analysis. **Results:** Mean age of participants was 28.7±2.6 years. Mean gestational age was 29.1±2.8 weeks. Mean cystatin C value were higher among cases (0.830±0.28 mg/L) than controls (0.167±0.19 mg/L). Cystatin C had 88% sensitivity and 98% specificity. Serum creatinine and uric acid had much less sensitivity and specificity as compared to cystatin C. **Conclusion:** Serum cystatin C is an important biomarker that more accurately captures early renal impairment compared to conventional indicators.

Keywords: Cystatin C, serum creatinine, uric acid

Pak J Physiol 2026;22(1):3–5, DOI: <https://doi.org/10.69656/pjp.v22i1.1866>

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INTRODUCTION

Pre-eclampsia is most common pregnancy related complication that is associated with maternal morbidity and mortality.¹ According to World Health Organization, 16.1% maternal deaths in developed countries are due to pre-eclampsia.² Worldwide, 76,000 maternal and 500,000 infant deaths each year are due to pre-eclampsia.³

Pre-eclampsia is a multisystem disorder characterized by blood pressure of 140/90 mmHg or more with proteinuria.⁴ According to American College of Obstetricians and Gynaecologists guidelines (ACOG), pre-eclampsia is established when maternal blood pressure is >140/90 mmHg measured on two intervals at least 6 hours apart and proteinuria >300 mg within 24 hours, after 20 weeks of gestation.⁵ Renal function derangement is an essential pathophysiological component of pre-eclampsia during pregnancy.⁶ Renal function requires close monitoring to avoid serious renal complications.⁷ Early diagnosis of renal function impairment and progression of pre-eclampsia during pregnancy is a great challenge for clinicians. It is worth stating that even in its current state of imperfection, cystatin C has still been found to provide a more accurate estimate of glomerular filtration rate (GFR) than creatinine in certain patient populations.⁸

Serum creatinine has been used as an important marker for GFR, but pregnancy related vasodilatation of renal vessels leads to changes in glomerular filtration rate thus limiting the use of serum creatinine as GFR marker. Another predictive GFR marker of pre-eclampsia is uric acid.⁹ It was considered as a good predictor in clinical observation due to increase in its concentration with severity of pre-eclampsia.¹⁰ However, some studies have reported uric acid as poor hypertensive disorder estimator.¹¹

Cystatin C is a new endogenous marker for assessment of GFR.¹¹ It is produced at constant rate in all nucleated cells and eliminated by glomerular filtration.⁸ Proteases such as cysteine proteases (cathepsins) are responsible for the degradation of extracellular matrix associated with the trophoblastic invasion seen in normal placental development.¹² Cystatin C is a member of the cystatin super family of cysteine protease inhibitors and is proved to be better GFR marker in individuals with small to moderate reduction in GFR.^{13,14} The maternal decidua is thought to control or limit the process of placental development by a coordinated expression of cystatin C.¹⁵ The finding of increased placental expression and higher serum concentrations of cystatin C in women with clinically evident symptomatic pre-eclampsia suggests that cystatin C may be involved in the aetiology of pre-eclampsia.¹⁶

Traditional markers of renal function are unable to assess renal impairment at an early stage in pre-eclampsia, hence the utility for an emerging renal function marker like cystatin C is suggested for early diagnosis and timely management. No local study has been carried out on the utility of this biomarker. This study was designed to evaluate diagnostic accuracy of serum cystatin C as an early marker of acute kidney injury in patients of pre-eclampsia.

METHODOLOGY

This case control study was conducted in Department of Chemical Pathology of a tertiary care hospital. The total duration of the study was 10 months from Jun 2024 to Apr 2025. Study was approved by ethical review committee and consent was taken from each participant.

A sample size of 70 (95% confidence interval) was calculated with 1:1 case to control ratio using WHO Sample Size Calculator. Participants were divided in two groups; cases (pregnant women with pre-eclampsia) and controls (healthy pregnant women). Simple consecutive sampling technique was used for distribution of participants into both groups. Urine analysis and blood pressure measurements were performed at the time of confirmation of pregnancy to avoid confounders like pre-existing proteinuria and renal disease. The inclusion criterion was based on gestation age ≥ 20 weeks and absence of concomitant disease in both groups. Cases were defined as pregnant ladies with systolic blood pressure (SBP) ≥ 140 mmHg and diastolic blood pressure (DBP) ≥ 90 mmHg, and proteinuria on dipstick (Roche diagnostics) checked in spot urine sample after 20th week of gestation. Controls were defined as healthy pregnant mothers with SBP < 140 mmHg and DBP < 90 mmHg without proteinuria. Mothers with affected glomerular filtration rate due to pre-gestational hypertension, diabetes mellitus, thyroid illness and other renal diseases confirmed by previous documents or medications were excluded from study. A questionnaire was used for other parameters like parity, weight, height, BMI, systolic and diastolic BP, gravidity etc.

A total of 5 mL blood was withdrawn from each participant. Two mL blood was taken in sodium fluoride tube for glucose estimation and 3 mL in plain gel tube for renal function tests, uric acid, cystatin C and thyroid stimulating hormone estimation. The sample was centrifuged at 4,000 rpm and tests were performed. Serum cystatin C, serum creatinine and uric acid were measured with particle enhanced immunoturbidimetric method, Jaffe's and standard enzymatic PAP method respectively on Selectra E.

Data were analysed using SPSS-22. Descriptive statistics (percentages, mean, SD) were used to describe the data. Pearson's correlation was applied for the correlation between creatinine and cystatin C among the cases. Independent *t*-test was applied for

comparison of cystatin C, creatinine and uric acid level in both groups. ROC analysis was used for diagnostic evaluation of cystatin C, creatinine and uric acid.

RESULTS

Total 70 participants were included in study. There were 35 (50%) patients of pre-eclampsia while 35 (50%) were healthy pregnant women. The mean age of cases was 26.7 ± 2.6 years and that of controls was 25.4 ± 2.3 years. Mean BMI was 24.6 ± 1.90 (Kg/m²) among cases, and 22.4 ± 1.44 (Kg/m²) in controls. Mean gestational age among all participants was 29.1 ± 2.8 weeks. Mean SBP was 134 ± 26.4 mmHg and mean DBP was 83.3 ± 13.7 mmHg was noted in controls while 145 ± 28.1 mmHg SBP and 97 ± 15.7 mmHg DBP was noted in cases.

The renal markers diagnostic evaluation using the receiver operating curve (ROC) showed that the sensitivity and specificity of cystatin C was 88% and 98%, while that of creatinine was 63% and 27.5%, and of uric acid was 79% and 71% respectively. Cystatin C had 98% positive predictive value (PPV), and 89% negative predictive value (NPV). Creatinine had 47% PPV, and 42.2% NPV, and uric acid had 66.7% PPV, 82% NPV. There was a positive correlation between cystatin C and creatinine among pre-eclamptic mothers ($r=0.4323$). ROC analysis showed better diagnostic accuracy of cystatin C with area under curve (AUC) of 0.9 compared to creatinine (0.4) and uric acid (0.8).

Table-1: Comparison of cystatin C, creatinine and uric acid between cases and controls (Mean $\pm$ SD)

Biochemical parameters	Cases (n=35)	Controls (n=35)	<i>p</i>
Cystatin C (mg/L)	0.830 $\pm$ 0.28	0.167 $\pm$ 0.19	<0.001
Creatinine (μ mol/L)	66.20 $\pm$ 7.4	63.91 $\pm$ 7.1	0.193
Uric acid (μ mol/L)	431.4 $\pm$ 48.9	271.3 $\pm$ 41.5	<0.001

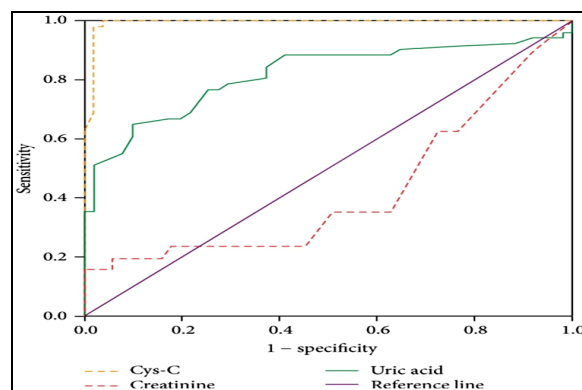


Figure-1: ROC showing diagnostic accuracy of cystatin C compared with other renal markers

DISCUSSION

Altered renal function is an essential component of the pathophysiological process in pre-eclampsia. The kidneys play a significant part in the turnover of most low molecular weight substances such as creatinine, urate and cystatin C.¹⁷ Bellos I *et al*¹⁸ reported a meta-

analysis which concluded that cystatin C seems to be a promising biomarker for the detection of pre-eclampsia during 3rd trimester of pregnancy with 85% sensitivity and 84% specificity. Our study had 88% sensitivity but 98% specificity. Padma Y *et al*¹⁹ concluded that maternal serum cystatin C, creatinine and uric acid were all significantly elevated at the end of pregnancy in pre-eclampsia compared to those of healthy pregnant women. Our study is in agreement to them. Wattanavaekin K *et al*²⁰ also suggested that cystatin C is a valid biomarker to predict AKI in pre-eclampsia.

Chew JS *et al*²¹ reported that despite the superior diagnostic accuracy of cystatin C compared with serum creatinine for detection of early renal impairment and also prediction of long-term outcomes, there is still a paucity of evidence that it actually leverages important clinical decisions more effectively than the use of serum creatinine alone or eGFR.

LIMITATIONS

The study was conducted with a small sample size at a single centre, hence the findings cannot be generalized.

CONCLUSION

Serum cystatin C seems to be a more specific biomarker for detection of AKI in pre-eclampsia during 3rd trimester of pregnancy. Further work is recommended to assess its predictive accuracy in early pregnancy and instituting appropriate management strategies.

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Received: 19 Jun 2025

Reviewed: 29 Jan 2026

Accepted: 8 Feb 2026

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AH: Diagnosis, data analysis

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SBB: Revision, script editing

SB: Literature search, bibliography

Conflict of Interest: No conflict of interest is declared,

Funding: No funds have been received from any agency



ORIGINAL ARTICLE

CORRELATION OF URINARY NEUTROPHIL GELATINASE ASSOCIATED LIPOCALIN WITH ERYTHROPOIETIN IN ACUTE KIDNEY INJURY

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Background: Neutrophil gelatinase-associated lipocalin (NGAL) and erythropoietin (EPO) are emerging biomarkers in acute kidney injury (AKI). While both markers are elevated independently in AKI, their correlation has not been explored. This study aimed to evaluate the correlation between urinary NGAL and serum EPO levels in patients with AKI. **Methods:** This comparative cross-sectional study was conducted at a tertiary care hospital in Peshawar. A total of 106 AKI patients (58 males, 48 females) aged over 18 years were enrolled through purposive sampling. Patients with chronic kidney disease, diabetes mellitus, hypertension, autoimmune diseases, or on long-term medications were excluded. AKI was staged using RIFLE criteria. Urinary NGAL and serum EPO levels were measured and compared across the severity stages. **Results:** Among stage 1 AKI patients, NGAL levels were 126 ± 43 ng/mL in females and 138 ± 37 ng/mL in males ($p=0.29$), while EPO levels were 82.8 ± 32 mIU/mL in females and 139 ± 74 mIU/mL in males ($p=0.002$). In stage 2 AKI, NGAL values were 116.1 ± 30 ng/mL in females, 135 ± 36 ng/mL in males ($p=0.15$), and EPO was 99 ± 65 mIU/mL in females and 102 ± 35 mIU/mL in males ($p=0.87$). Stage 3 AKI cases had NGAL 117.5 ± 41 ng/mL in females and 126 ± 42 ng/mL in males ($p=0.52$), and EPO 131 ± 69 mIU/mL in females and 117 ± 57 mIU/mL in males ($p=0.50$). **Conclusion:** Except in stage 1 AKI, no significant correlation was found between urinary NGAL and serum erythropoietin levels. Disease history and duration, glomerular filtration rate, or lack of serial biomarker measurements may cause results variability.

Keywords: Acute kidney injury, Biomarkers, Erythropoietin, Nephrotoxicity, NGAL, Renal failure

Pak J Physiol 2026;22(1):6–8, DOI: <https://doi.org/10.69656/pjp.v22i1.1891>

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INTRODUCTION

Acute Kidney Injury (AKI) is a critical clinical syndrome characterized by a rapid decline in glomerular filtration rate, leading to impaired excretion of metabolic waste.¹ It is associated with an increased risk of cardiovascular events, progression to chronic kidney disease, and high mortality.² The diagnosis of AKI relies on clinical investigations, primarily serum creatinine levels and urine output, which assess kidney function rather than direct kidney injury.³ However, these markers have limitations, as they do not predict adverse outcomes, hospital stay, treatment costs, or mortality, nor do they identify the underlying cause of AKI.⁴ To improve diagnostic accuracy and management, reliable biomarkers indicative of kidney injury has been identified in blood and urine. These biomarkers, when used alongside conventional markers, enhance early detection and treatment strategies. Erythropoietin (EPO) exhibits renoprotective properties, potentially mitigating AKI progression.⁵

Timely diagnosis and management are essential for optimal AKI prognosis. Serum creatinine, a delayed and nonspecific marker of kidney injury, limits early detection.⁶ Neutrophil gelatinase-

associated lipocalin (NGAL), a glycoprotein of the Lipocalin 2 family, is an early and sensitive biomarker, significantly upregulated in ischemic and nephrotoxic AKI, with detectable levels in blood and urine.⁷ NGAL expressed from the tubular epithelial cell and tubular epithelium of the distal nephron after damage, therefore increase NGAL in the urine and serum reflect kidney disease. Following damage to the kidneys, especially the tubular epithelium of the distal nephron, NGAL is released from tubular epithelial cells, leading to elevated levels in both urine and serum.⁸ Therefore, increased NGAL concentrations in these fluids are indicative of kidney disease.⁹

Erythropoietin (EPO), induced by hypoxia, exhibits renoprotective properties through anti-apoptotic and regenerative mechanisms.^{10,11} There is impaired production of NGAL and EPO in AKI patients in different studies^{8,12}, however, literature yield no study of its kind to find out the levels of both these markers in the same patients. Correlation between the two markers may enable us to evaluate and treat AKI efficiently. This study investigated the correlation between urinary NGAL and EPO to enhance early AKI diagnosis and management, potentially alleviating patient burden.

METHODOLOGY

This comparative cross-sectional study was conducted after getting approval from the Institutional Research and Ethical Board vide certificate No. DIR/KMU-AS&RB/CU/001747. Ethical approval was obtained from IBMS, Khyber Medical University via diary No. 2022/9298 along with approvals from Hayatabad Medical Complex via diary No. 7413/2022 and Khyber Teaching Hospital via diary No. 4219/2022. Data was collected from Institute of Kidney Diseases, Hayatabad Medical Complex, and Khyber Teaching Hospital, Peshawar.

Keeping the confidence interval of 95%, prevalence of 20% and power of 80, the sample size was calculated as 106. In total about 800 patients were screened, out of which 106 patients fulfilling the criteria were selected for the study. Written informed consent was taken along with identity details from all patients. Adults aged above 18 years with acute onset of symptoms, Azotemia (deranged BUN), and all forms pre-renal, renal, and post-renal cases were included. Chronic Kidney Disease patients, established kidney disease patients due to Diabetes Mellitus, Hypertension, those taking any drugs, patients with autoimmune disorders were excluded.

Anthropometric measurements and blood and urine samples from the patients were collected. The level of NGAL and EPO was determined using Enzyme-linked immunosorbent assay (ELISA).

Data were analysed using SPSS-24. Normality of data was determined. Quantitative data were presented as Mean $\pm$ SD. Male to female differences were determined through independent sample *t*-test. Correlation between NGAL and EPO was determined through Pearson and Spearman correlation, and $p \leq 0.05$ was considered as statistically significant.

RESULTS

Table-1 presents baseline data of enrolled patients showing the total sample size, mean age and BMI.

Table-2 shows levels of severity for the 3 stages of AKI. Erythropoietin levels were significantly higher in males in stage 1 AKI than in females ($p=0.002$). Other parameters showed no significant gender differences.

Table-3 presents correlation analysis among different test parameters. NGAL shows negative correlation with urinary WBCs, while rest of the parameters shows no significant correlation.

Table-1: Demographics of selected individuals

Demographics	Mean values	Female (Mean $\pm$ SD)	Male (Mean $\pm$ SD)	<i>p</i>
Sample size (n)	106	48	58	
Age (Years)	51.1	48.92 $\pm$ 18.51	52.93 $\pm$ 20.88	0.3
BMI (Kg/m ²)	23.9	24.50 $\pm$ 1.97	23.56 $\pm$ 2.23	0.02

Table-2: NGAL and EPO levels according to severity of AKI in males and females

Severity of AKI	Total (n=106) Mean	Male (n=58) (Mean $\pm$ SD)	Female (n=48) (Mean $\pm$ SD)	<i>p</i>
Stage 1 (n)	45	24	21	
NGAL (ng/mL)	132	138 $\pm$ 37	126 $\pm$ 43	0.29
EPO (mIU/mL)	112	139 $\pm$ 74	83 $\pm$ 32	0.002
Stage 2 (n)	27	16	11	
NGAL (ng/mL)	127	135 $\pm$ 36	116 $\pm$ 30	0.15
EPO (mIU/mL)	100	102 $\pm$ 35	99 $\pm$ 65	0.87
Stage 3 (n)	34	18	16	
NGAL (ng/mL)	122	126 $\pm$ 42	118 $\pm$ 41	0.52
EPO (mIU/mL)	124	117 $\pm$ 57	131 $\pm$ 69	0.50

Table-3: Correlation among different variables

	Age	Level of Severity	BMI	Urinary WBCs	EPO	NGAL
Age	<i>r</i>	1	-0.19*	-0.08	0.10	0.00
	<i>p</i>		0.04	0.40	0.28	0.96
Level of Severity	<i>r</i>		1	0.04	-0.01	0.07
	<i>p</i>			0.63	0.86	0.47
BMI	<i>r</i>			1	0.12	0.11
	<i>p</i>				0.20	0.25
Urinary WBCs	<i>r</i>				1	0.10
	<i>p</i>					0.29
EPO	<i>r</i>					1
	<i>p</i>					0.19
NGAL	<i>r</i>					
	<i>p</i>					

DISCUSSION

Our findings showed that males were having high severity of acute kidney injury in all three stages which may be evident by males having more exposure to nephrotoxicity, trauma like road traffic accidents, nephrotoxic radiation work routines along with drugs including smoking and others, while females also subjected to the same as males were found less susceptible to either may be less exposure to trauma or usage of nephrotoxic drugs. Hormones and chromosomes differences in males and females also affect kidneys functions in both genders. These differences affect body response to blood flow, inflammation, and protection against cell damage, especially during kidney injury. Some of these differences are always present, while others happen in response to injury.¹³ One study suggests that in mice, the oestrogen helps protect the kidney's filtering barrier during a type of kidney injury caused by reduced blood flow and then its return (ischemia/reperfusion injury).¹⁴ Oestrogen helps widen blood vessels by increasing nitric oxide, while testosterone can reduce this effect. In cases of AKI, females are generally more resistant to inflammation. In some studies, oestrogen has been shown to reduce the activity of immune cells like lymphocytes and neutrophils, which play a key role in causing kidney damage during AKI.¹⁵

The concentration of urinary white blood cells was found to be associated with low levels of NGAL in our study population which can be a part of multiple

scenarios as in AKI patients, urinary infection is common or can lead to acute tubular necrosis and ultimately AKI. The NGAL seems to be influenced by urinary WBCs and it is reported that the urinary NGAL may be elevated with pyuria because neutrophils contain NGAL. The negative correlation may be in part that urine NGAL can stay elevated up to 7 days after the AKI has developed and levels decrease down after or displacement of the NGAL by the white blood cells.¹⁶

Erythropoietin levels show no significant association with the level of severity of AKI and with urinary NGAL at any stage of AKI in our study. Previous studies also reported that EPO concentrations cannot discriminate the severity of critical illness in ICU patients.¹⁷ Some studies also showed negative correlation between EPO and NGAL in chronic kidney disease patients.⁹

CONCLUSION

Except in stage 1 AKI, no significant correlation was found between urinary NGAL and serum erythropoietin levels. Erythropoietin may not play a direct role in influencing NGAL levels or *vice versa* in AKI. Disease history and duration, glomerular filtration rate, or lack of serial biomarker measurements may cause results variability.

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Received: 20 Aug 2025

Reviewed: 19 Jan 2026

Accepted: 8 Feb 2026

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Conflict of Interest: None to declare

Funding: This study was supported by ORIC Department of Khyber Medical University, Peshawar



ORIGINAL ARTICLE

ANTIHYPERTENSIVE EFFECTS OF ETHANOLIC EXTRACT OF *TRAPA NATANS* SEEDS IN L-NAME-INDUCED HYPERTENSIVE RATS

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Background: There is need for safer and cost-effective therapeutic intervention for hypertension. *Trapa natans* exhibits antioxidant and anti-inflammatory activity. However, its efficacy as an antihypertensive has not yet been established. This study aimed to examine the potential antihypertensive effect of ethanolic extract of *Trapa natans* seed in L-N^G-Nitro Arginine Methyl Ester (L-NAME) induced hypertensive rats. **Methods:** This study used 30 rats which were divided into six groups of five rats each. The control group was given 5 mg/Kg normal saline. L-NAME and captopril (20 mg/Kg) were given to the second group. L-NAME and different doses of *Trapa natans* were given to 3rd, 4th, 5th and 6th groups. Non-invasive blood pressure was checked weekly. On the 29th day, the invasive blood pressure of the animals was checked. **Results:** The results showed that the non-invasive blood pressure altered substantially across the groups over time, especially the dose of 400 mg/Kg *Trapa natans* notably reduced the blood pressure. By the end of 28 days the invasive blood pressure also showed a noteworthy reduction in blood pressure for the dose of 400 mg/Kg *Trapa natans*. **Conclusion:** This study supports the beneficial ability of *Trapa natans* in lessening L-NAME induced hypertension. Its effects are likely to be caused by its phytochemical constituent, which demonstrates strong antioxidants and anti-inflammatory activities of *Trapa natans*.

Keywords: Hypertension, Invasive blood pressure, L-NAME, Non-invasive blood pressure, *Trapa natans*

Pak J Physiol 2026;22(1):9–11, DOI: <https://doi.org/10.69656/pjp.v22i1.1888>
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INTRODUCTION

Hypertension is a chronic disease of the cardiovascular system that is characterized by consistently high blood pressure in the arteries, which is determined by systolic blood pressure (SBP) 140 mmHg and/or diastolic blood pressure (DBP) 90 mmHg.¹ It is a significant global health issue and the principal risk factor to cardiovascular morbidity and mortality.

The WHO claims that hypertension causes about 9.4 million deaths every year around the globe and over 1.28 billion adults aged between 30 and 79 years are affected.² It is raising the rate of morbidity and mortality.³ Nearly 64% of stroke patients have hypertension.⁴ Approximately half of its patients never get a diagnosis, especially in low- and middle-income countries because their awareness of the disease and access to healthcare services are less. The current trends in lifestyles and demographic shifts have also contributed to the fact that prevalence of hypertension has grown significantly throughout the last several decades globally.²

Prevalence of hypertension is 31.5% in low- and middle-income countries and 28.5% in high-income countries.⁵ Countries with high population densities and low and moderate income are more susceptible to hypertension. Hypertension is one of the serious health burdens in Pakistan and prevalence of the conditions has been reported as 21.6% in urban population and 16.2% in rural population.⁶ Almost three-quarters of people with

hypertension in Pakistan do not even know that they have the disease.⁷ Regional differences have also been noted as women are suffering more in Khyber Pakhtunkhwa and Baluchistan, and men are found to be more prevalent in the Punjab and Sindh.^{6,8}

Risk factors of hypertension can be categorized in the broad scope as non-modifiable factors, e.g., age, sex, and inherited genetic makeup, and modifiable factors, e.g., excessive salt intake in the diet, obesity, inactivity, smoking, and alcoholism.⁸ The pathophysiology of hypertension is linked to dysregulation of rennin-angiotensin-aldosterone system (RAAS), hyperactivity of the sympathetic nervous system, endothelial dysfunction, and a lack of nitric oxide bioavailability.⁹

Despite existence of various pharmacological classes like diuretics, angiotensin-converting enzyme inhibitors, calcium channel blockers, and β -adrenergic antagonists to manage hypertension, prolonged intake of these drugs is known to cause complications such as electrolyte imbalance, gastrointestinal upsets, and cardiovascular complications.⁹ As a result, research on antioxidant and anti-inflammatory properties of plant-based therapies has become of interest. It is approximate that 75–80% of the world population depends, partially, on herbal medicines as primary healthcare.¹⁰

Aquatic polyphenol-flavonoid-rich plant, *Trapa natans*, has been shown to be useful in antioxidant and anti-inflammatory processes. Nevertheless, its

antihypertensive effect has not been properly investigated. The aim of current work was to determine antihypertensive properties of ethanolic extract of *Trapa natans* seeds on L-N^G-Nitro Arginine Methyl Ester (L-NAME), (a non-selective nitric oxide synthase inhibitor widely used to induce experimental hypertension by reducing nitric oxide bioavailability and increasing vascular resistance) induced hypertensive rat model.

MATERIAL AND METHODS

This study was conducted in June 2024 in the University of Lahore after approval of Ethical Review Board of Pharmacy Department vide IREC No. IREC-2023-54. The laws of the National Institute of Health regarding animal experiments were adhered to in this study.

The experiment lasted for 28 days. Rats were randomly divided into 6 groups, i.e., control, L-NAME hypertensive control, L-NAME plus captopril (20 mg/Kg), and L-NAME plus *Trapa natans* at doses of 100 (T-100), 200 (T-200), and 400 (T-400) mg/Kg.

Both non-invasive and invasive blood pressure measurements were carried out. The non-invasive blood pressure was measured every week, with rats being acclimated to a comfortable, quiet, and dark atmosphere and restrained to reduce cases of stress as much as possible.¹¹ The tail was warmed and tail cuff was used to feel the pulse with a photoplethysmograph to stimulate a pressure transducer. In the invasive measurements, carotid cannulation was done following anaesthesia with diazepam and ketamine.^{12,13} The neck was shaved and disinfected, and under close dissection the carotid artery and trachea were revealed. A cannula filled with heparin saline was inserted into the carotid artery, fastened with clamps and threads and attached to a pressure transducer, with the monitoring of bleeding.

The Mean±SEM was used to express the data. In order to determine the level of significance between the control and experimental groups, the data obtained were analyzed through two-way analysis of variance (ANOVA) with post hoc Bonferonni test, and $p \leq 0.05$ was considered as statistically significant.

RESULTS

Daily administration of L-NAME resulted in rise in SBP from 135±4 mmHg in week 1 to 208±10 mmHg by week 4. *Trapa natans* treatment induced an antihypertensive effect in dose dependence manner. The T-100 group recorded a reduction of 13.75 mmHg (9.34%) by week 4, T-200 had a reduction of 23.55 mmHg (19.5%) and T-400 recorded a reduction of 48.30 mmHg (42.58%), which was similar to captopril (48.4 mmHg, 43.26%) and the control group (42.0 mmHg).

Bonferonni was used to determine the group significantly different in comparison to L-NAME group. As there was a constant change in blood pressure, L-NAME group had the greatest increase. Blood pressure

of the T-100 and T-200 groups revealed a constant increase although it started to decline gradually. T-400 progressively presented a decrease in blood pressure, indicating an antihypertensive effect. (Table-1).

Invasive blood pressure measurement in all groups differed. The mean difference, however, revealed that doses of 100 and 200 mg/Kg of *Trapa natans* are less effective as no differences were observed; the 400 mg/Kg dose of *Trapa natans* showed significant differences in the invasive blood pressure after 28 days. The results demonstrate that such changes were similar to those caused by captopril. (Table-2).

Table-1: Non-invasive blood pressure of animals at weekly intervals (mmHg, Mean±SE)

Group	Week 1	Week 2	Week 3	Week 4
Control	130±3	131±4	130±4	131±4
L-NAME	135±4	158±6	186±8	208±10
Captopril	128±2	129±3	121±3	118±3
T-100	132±3	148±4	166±5	189±6
T-200	129±3	140±3	160±4	167±4
T-400	127±3	128±3	123±3	115±4

Table-2: Mean invasive blood pressure (IBP) after 28 days of treatment compared to L-NAME group (mmHg, Mean±SE)

Group	Mean IBP	Change in BP	p
Control	125±4	N/A	N/A
L-NAME	175±7	---	N/A
Captopril	110±3	-65	<0.001*
T-100	165±5	-10	NS
T-200	145±3	-30	<0.01*
T-400	115±3	-60	<0.001*

*Significant

A dose-dependent response was seen among the test compound-treated groups; T-100 showed an increase of 14.40 mmHg ($p=0.0137$), T-200 showed a larger effect of 37.20 mmHg ($p=0.000$), and T-400 produced an increase of 66.00 mmHg ($p=0.000$), which was almost identical to the effect of captopril.

DISCUSSION

One of the most important physiological indicators of cardiovascular functioning is blood pressure, and its proper measurement is crucial with experimental hypertension models.^{14,15} Both non-invasive and invasive techniques were used to assess the antihypertensive effect of *Trapa natans* in L-NAME induced hypertensive rats. The results of both approaches were consistent.^{14,16}

With the non-invasive measurements, it was demonstrated that *Trapa natans* at the dose of T-400 generated significant and enduring effect of alleviating blood pressure during treatment. Notably, the extent of pressure drop on this dose was similar to captopril indicating clinically useful antihypertensive effect.¹³ Weaker doses (T-100 and T-200) also had blood pressure-lowering activity, but the relatively small size of the effects shows that there is a dose-dependent effect

as opposed to identical therapeutic effect.¹⁷ This was further confirmed by invasive blood pressure measurements.

The invasive data proved that *Trapa natans* had a salient effect to reduce hypertension in L-NAME-treated rats with T-400 dose showing the strongest effect. The stability of the antihypertensive effect of *Trapa natans* is emphasized by the consistency between dynamic monitoring of blood pressure by non-invasive methods and terminal measurements reported by invasive method, and accuracy of tail-cuff method to determine the tendency of a trend.^{15,16}

The combined analysis of invasive and non-invasive results suggests that *Trapa natans*, especially with higher levels of dosing, possesses a great potential of antihypertensive effect in L-NAME-induced hypertension. Such findings indicate its potential therapeutic value and need to explore its pharmacology and safety in the long term.

CONCLUSION

Trapa natans has an antihypertensive effect in L-NAME induced hypertension in Wistar rats. There is a definite dose-ranging effect with the T-400 dose causing a significant decrease in blood pressure similar to the conventional antihypertensive medication, captopril. Smaller dosage (T-100 and T-200) had a moderate effect, and it is another indicator of criticality of dosage.

RECOMMENDATIONS

Future research ought to use a wider dose to determine the therapeutic window and explain the dose response relationship of *Trapa natans*. An exploration of its molecular mechanisms, especially the nitric oxide regulation, oxidative stress, and vascular protection, should be investigated. It is suggested to use chronic hypertension models in long-term studies to determine safety and long-term effectiveness. Further investigation of *Trapa natans* can be conducted through separation of bioactive compounds to place *Trapa natans* in pharmacological context and use in humans.

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Received: 10 Aug 2025

Reviewed: 11 Jan 2026

Accepted: 13 Jan 2026

Contribution of Authors:

Authors approved the draft and are accountable in ensuring that questions related to accuracy or integrity of the work are duly investigated and resolved.

ML: Concept and study design, data acquisition, 1st draft **KM:** Critical review and final approval **MNM:** Critical review and final approval

HS: Data collection and compilation **SHSH:** Data analysis and tabulation **AA:** Data collection and compilation

Conflict of Interest: No conflict of interest is declared, **Funding:** No funds have been received from any agency



ORIGINAL ARTICLE

PROTECTIVE ROLE OF EMPAGLIFLOZIN AGAINST METHOTREXATE-INDUCED LIVER INJURY

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Background: Methotrexate (MTX), a commonly used chemotherapeutic and immunosuppressive agent, is associated with dose-dependent hepatotoxicity characterized by oxidative stress and liver tissue damage. Empagliflozin (EM), a sodium-glucose cotransporter-2 (SGLT2) inhibitor, has shown antioxidant properties in various organ systems. This study aimed to evaluate the hepatoprotective effects of EM against MTX-induced liver injury in rats. **Methods:** Forty adult Wistar rats were divided into four groups of 10 each. Group A (Control, 5% dimethyl sulfoxide as vehicle control), Group B (MTX 20 mg/Kg, i.p., on day 3), Group C (EM 10 mg/Kg/day for 7 days+MTX), and Group D (EM 30 mg/Kg/day for 7 days+MTX). Serum liver enzymes (ALT, AST), bilirubin, and oxidative stress markers (MDA, SOD, GPx, catalase) were measured. Liver tissues were also examined histologically using a semi-quantitative scoring system for cellular degradation, cytoplasmic vacuolization, sinusoidal dilatation, and inflammatory infiltration. **Results:** MTX administration resulted in significant elevations in ALT, AST, bilirubin, and MDA levels, along with reductions in SOD, GPx, and catalase activities ($p<0.001$). Histopathology confirmed severe hepatic damage in the MTX group. EM co-treatment, significantly increased antioxidant enzyme activity, reduced lipid peroxidation, and improved histological scores, with Group D showing better results ($p<0.001$). Biochemical and microscopic parameters showed a dose-dependent protective effect of EM. **Conclusion:** EM significantly attenuates MTX-induced hepatotoxicity by reducing oxidative stress and preserving liver histoarchitecture. These findings suggest a potential role for EM as a hepatoprotective agent against drug-induced liver injury.

Keywords: Empagliflozin, Hepatotoxicity, Liver injury, Methotrexate, Oxidative stress

Pak J Physiol 2026;22(1):12–5, DOI: <https://doi.org/10.69656/pjp.v22i1.1886>

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INTRODUCTION

Drug-induced hepatic injury has become a widespread and significant global health issue.¹ This challenge not only hinders drug approval but also negatively impacts the therapeutic effectiveness of drugs and reduces patient compliance. Drug-induced liver toxicity may arise through several mechanisms, including direct interference with cellular processes, formation of toxic metabolites, or immune-mediated responses that provoke inflammation and hepatocellular damage.² In recent years, the occurrence of hepatotoxic reactions due to medications has increased, prompting growing concern in clinical and pharmaceutical research communities.³

Methotrexate (MTX) is an antimetabolite and folic acid analogue commonly used to manage malignancies like leukaemia and chronic inflammatory disorders such as psoriasis and rheumatoid arthritis.⁴ Its clinical application is limited by adverse effects, particularly hepatotoxicity. Although the precise pathways remain under investigation, oxidative stress and the generation of reactive oxygen species (ROS) are thought to play central roles in MTX-induced hepatic injury.^{5,6} By disrupting mitochondrial respiration, MTX

promotes excessive ROS production, which can damage cellular structures, initiate lipid peroxidation, and trigger hepatocyte death.⁷ Therefore, minimizing oxidative stress and ROS accumulation is a key target for preventing MTX-related liver damage.

Empagliflozin (EM) is a sodium-glucose cotransporter-2 (SGLT-2) inhibitor that lowers blood glucose by blocking glucose and sodium reabsorption in the proximal renal tubules.⁸ SGLT-2 facilitates the reabsorption of glucose and sodium from urine into the bloodstream. Although it is primarily expressed in the kidneys, it is also found in the liver, heart, thyroid, and skeletal muscle.⁹ In addition to its glucose-lowering effect, empagliflozin has demonstrated pleiotropic benefits, including reductions in body weight, blood pressure, arterial stiffness, lipid levels, systemic inflammation, insulin resistance, and uric acid concentration factors that contribute to cardiovascular protection.¹⁰ Emerging evidence indicates that EM has protective effects on the kidneys, liver, gastrointestinal system, brain, and peripheral nerves.^{11–13} These protective effects are largely attributed to its ability to reduce oxidative stress. The objective of the current study was to evaluate the hepatoprotective effects of empagliflozin against methotrexate-induced liver injury.

MATERIAL AND METHODS

This study was conducted in the Department of Pathology, Isra University, Hyderabad, between February and July 2025, following approval from the Institutional Ethical Review Committee. A total of 40 healthy male Wistar rats (weighing 200±20 g) were procured. The sample size was determined using power analysis methods appropriate for animal research studies.¹⁴ Rats were kept in an appropriate standard habitat with a regular photoperiod (12/12 hours light/dark cycle).

All rats had regular food and unlimited access to water. The animals were randomly allocated into 4 groups of 10 each. Group A (Control): Received 5% dimethyl sulfoxide (DMSO) orally by gavage once daily for 7 days as the vehicle control. Group B (MTX-only): Received 5% dimethyl sulfoxide (DMSO) orally by gavage once daily for 7 days, and a single intraperitoneal injection of methotrexate (20 mg/Kg body weight) on day 3. Group C (EM 20 mg/Kg+MTX): Received empagliflozin at a dose of 20 mg/Kg body weight, administered orally by gavage once daily for 7 days. Methotrexate (20 mg/Kg body weight) was administered as a single intraperitoneal injection on day 3. Group D (EM 30 mg/Kg+MTX): Received empagliflozin at a dose of 30 mg/Kg body weight, administered orally by gavage once daily for 7 days, with methotrexate (20 mg/Kg body weight) given as a single intraperitoneal injection on day 3.

Blood was collected through cardiac puncture post-cervical dislocation, following standard protocols. Serum levels of liver function markers (Alanine Transaminase, Aspartate Transaminase, Bilirubin), along with oxidative stress markers (Superoxide Dismutase, Glutathione peroxidase, Catalase, Malondialdehyde), were assessed using commercially available diagnostic kits.

Excision of liver tissue specimen, formalin fixation, and preparation of slides after H&E staining for microscopic evaluation was performed as per standard protocols. Histopathological evaluation was performed by an experienced pathologist who was blinded to the experimental groups. Liver sections were assessed using a semi-quantitative scoring system evaluating cellular

degradation, cytoplasmic vacuolization, sinusoidal dilatation, and inflammatory cell infiltration, graded on a scale from 0 (normal) to 3 (severe).¹⁵ Inter-observer variability was not assessed in this study.

Data was analyzed using SPSS-25, and $p<0.05$ was considered statistically significant.

RESULTS

Significant differences were observed among the groups for liver function markers. ALT levels were lowest in Group A (29.31±2.4 U/L) and highest in Group B (253.3±4.2 U/L), with Group C (130.2±4.9 U/L) and Group D (108.3±1.9 U/L) showing intermediate values ($p<0.001$). AST levels were significantly elevated in Group B (170.57±8.4 U/L) compared to Group A (38.42±3.2 U/L), while Group C (113.33±5.8 U/L) and Group D (94.81±7.6 U/L) showed partial recovery ($p<0.001$). Serum bilirubin was markedly increased in Group B (2.6±0.6 mg/dL) relative to Group A (0.8±0.1 mg/dL), whereas Group C (1.5±0.15 mg/dL) and Group D (1.1±0.11 mg/dL) demonstrated dose-dependent improvement ($p<0.001$). (Table-1).

Significant alterations were noted across all groups for antioxidant enzymes and lipid peroxidation. SOD levels were highest in Group A (418±32.57 U/mg) and significantly reduced in Group B (247.35±41.75 U/mg), while partial restoration was observed in Group C (330.14±24.71 U/mg) and Group D (367.78±18.97 U/mg) ($p<0.001$). GPx activity followed a similar trend, with the lowest levels in Group B (4.1±0.34 U/mg) and progressive improvement in Group C (5.6±0.47 U/mg) and Group D (6.3±0.25 U/mg), compared to Group A (7.2±0.52 U/mg) ($p<0.001$). Catalase levels were significantly reduced in Group B (32.54±3.48 U/mg) compared to Group A (55.31±4.22 U/mg), but improved in Group C (42.28±4.67 U/mg) and Group D (48.87±5.48 U/mg) ($p<0.001$). MDA levels, a marker of lipid peroxidation, were significantly elevated in Group B (330.57±21.34 nmol/mg) compared to Group A (160.44±20.41 nmol/mg), but decreased in Group C (261.72±16.18 nmol/mg) and further in Group D (202.62±21.88 nmol/mg) ($p<0.001$), indicating a dose-dependent reduction in oxidative damage. (Table-2).

Table-1: Liver function parameters (ALT, AST, and Bilirubin) across experimental groups

Variables	Group A	Group B	Group C	Group D	p
ALT (U/L)	29.31±2.40 ^{bcd}	253.30±4.20 ^{acd}	130.20±4.90 ^{abd}	108.30±1.90 ^{abc}	<0.001 *
AST (U/L)	38.42±3.20 ^{bcd}	170.57±8.40 ^{acd}	113.33±5.80 ^{abd}	94.81±7.60 ^{abc}	<0.001 *
Bilirubin (mg/dL)	0.80±0.10 ^{bcd}	2.60±0.60 ^{acd}	1.50±0.15 ^{abd}	1.10±0.11 ^{abc}	<0.001 *

*Statistically significant

Table-2: Oxidative stress markers (SOD, GPx, Catalase, and MDA) across experimental groups

Variables	Group A	Group B	Group C	Group D	p
SOD (U/mg)	418.00±32.57 ^{bcd}	247.35±41.75 ^{acd}	330.14±24.71 ^{abd}	367.78±18.97 ^{abc}	<0.001 *
GPx (U/mg)	7.20±0.52 ^{bcd}	4.10±0.34 ^{acd}	5.60±0.47 ^{abd}	6.30±0.25 ^{abc}	<0.001 *
Catalase (U/mg)	55.31±4.22 ^{bcd}	32.54±3.48 ^{acd}	42.28±4.67 ^{abd}	48.87±5.48 ^{abc}	<0.001 *
MDA (nmol/mg)	160.44±20.41 ^{bcd}	330.57±21.34 ^{acd}	261.72±16.18 ^{abd}	202.62±21.88 ^{abc}	<0.001 *

*Statistically significant

Semi-quantitative analysis of liver tissue revealed significant differences in histopathological damage among the groups. Group A exhibited no signs of liver injury, with a score of 0 for all parameters, including cellular degradation, cytoplasmic vacuolization, sinusoidal dilatation, and inflammatory cell recruitment. Group B showed the most severe damage, scoring 3 for all four parameters, indicating extensive hepatocellular injury, vacuolization, sinusoidal congestion, and prominent inflammation. Group C showed moderate improvement, with scores of 2 across all parameters, reflecting partial hepatoprotection. Group

D demonstrated the most marked improvement among treated groups, with scores of 1 for cellular degradation, cytoplasmic vacuolization, and inflammation, and 0 for sinusoidal dilatation, suggesting near-complete restoration of liver architecture. (Table-3).

Table-3: Semi-quantitative histopathological scores of liver tissue damage across experimental groups

Variables	Group A	Group B	Group C	Group D
Cellular degradation	0	3	2	1
Cytoplasmic vacuolization	0	3	2	1
Sinusoidal dilatation	0	3	2	0
Inflammatory cell recruitment	0	3	2	1

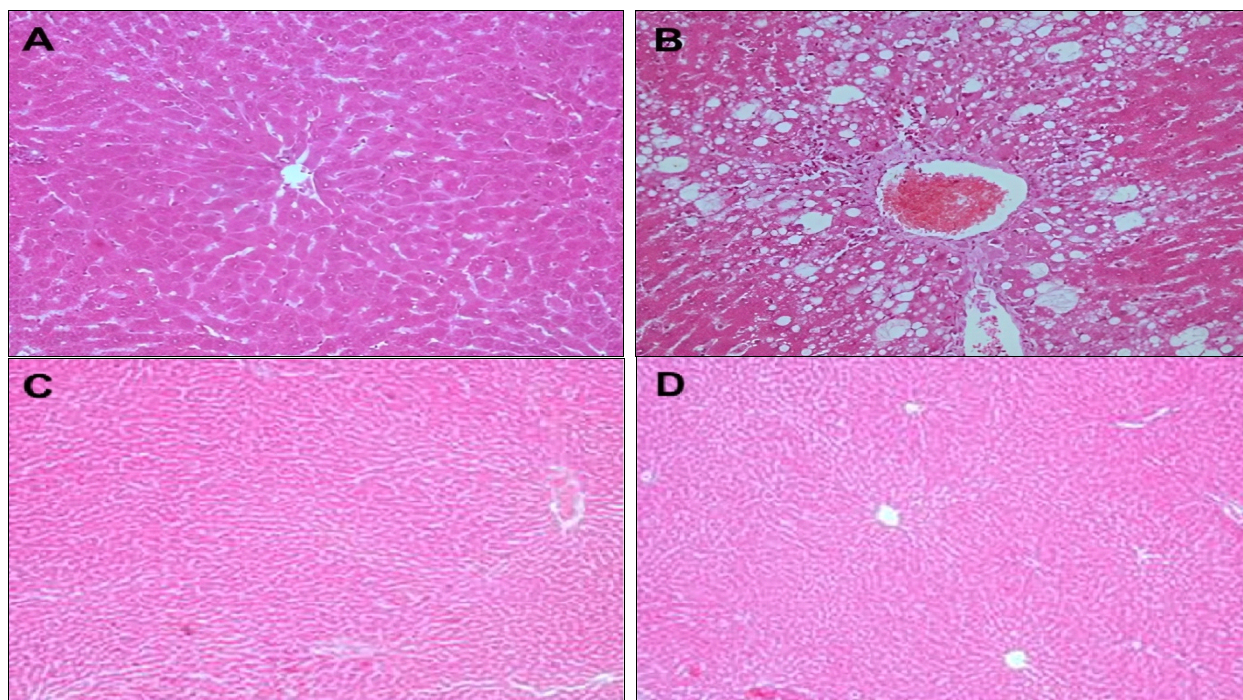


Figure-1: Histopathological evaluation of liver tissue in experimental groups A, B, C, D. (H&E stain, ×100)

DISCUSSION

Methotrexate, widely used in high doses for treating conditions such as acute leukaemia and severe psoriasis, is associated with hepatotoxicity, including acute liver injury, hepatic fibrosis, and cirrhosis.⁴ We investigated the protective effects of empagliflozin (EM) against MTX-induced liver damage. Our findings demonstrate that EM administration significantly attenuated hepatic injury, as reflected by improvements in both biochemical markers and histopathological features.

MTX-treated rats exhibited marked elevations in serum ALT, AST, and bilirubin levels —cytosolic chemicals that serve as sensitive indicators of hepatocellular damage and membrane integrity. This is consistent with the findings of Yanaşoğlu *et al*¹⁶, who reported MTX administration caused hepatotoxicity which was evident by the elevation of serum ALT, AST, and bilirubin levels. MTX significantly increased malondialdehyde (MDA) levels and reduced the activity

of antioxidant enzymes in liver tissue. Previous studies have shown that MTX promotes reactive oxygen species (ROS) formation, impairs NADPH availability, and depletes intracellular glutathione (GSH), thereby compromising the cellular antioxidant system and increasing susceptibility to oxidative damage.¹⁶ These biochemical findings were supported by histological evidence of MTX induced hepatocellular degeneration, cytoplasmic vacuolization, sinusoidal dilatation, and inflammatory infiltration, consistent with findings reported by Yanaşoğlu *et al*¹⁶ and Kalantari *et al*¹⁷.

EM pre-treatment notably reversed these alterations in a dose-dependent manner, with the higher dose demonstrating near-complete normalization of liver architecture and enzyme levels. EM's protective role may be attributed to its ability to enhance antioxidant enzyme activity and inhibit lipid peroxidation. In our study, EM significantly reduced MDA levels and restored GPx and SOD activities,

consistent with these prior findings.¹⁶ Histopathological improvements further confirmed the biochemical restoration, highlighting EM's ability to preserve hepatocyte integrity.

The reduction in ALT and AST levels in EM-treated groups aligns with earlier studies where EM attenuated liver enzyme elevations induced by MTX.¹⁷ Microscopic examination corroborated these results, revealing significant histological improvement in EM-treated rats compared to the MTX group. These findings suggest that EM may mitigate MTX-induced hepatotoxicity through antioxidant modulation.

A limitation of our study is the absence of inflammatory cytokine profiling, which could have further elucidated the anti-inflammatory effects of EM. Future studies should explore this aspect to better characterize the full spectrum of EM's hepatoprotective mechanisms.

CONCLUSION

Empagliflozin significantly protects rats' livers from damage caused by methotrexate. EM successfully reduced biochemical and histological indicators of hepatotoxicity by lowering oxidative stress and increasing antioxidant enzyme activity. These results demonstrate its potential as a preventative measure against drug-induced liver damage and call for additional research to determine its clinical suitability.

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Received: 19 Jul 2025

Reviewed: 7 Sep 2025

Accepted: 7 Sep 2025

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FRM: Drafting the article, collection and assembly of data

UM: Collection and assembly of data

SA: Drafting of script, critical analysis

FM: Drafting the article, collection and assembly of data

AGM: Drafting of article, literature review

Conflict of Interest: None to declare

Funding: None received



ORIGINAL ARTICLE

RELATIONSHIP OF EATING DOMAINS WITH ANXIETY AND PHYSICAL ACTIVITY AMONG UNIVERSITY STUDENTS OF LAHORE

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Background: The transition to higher education can significantly impact students' physical and mental health. Three dimensions of an eating behaviour: self-restraint, uncontrolled (binge) eating, and emotional eating are measured via Three-Factor Eating questionnaire. The objective of study was to explore the relationship of eating domains with anxiety and physical activity among university students.

Methods: This cross-sectional study was conducted from Apr to Oct 2024 in universities of Lahore. The study populace comprised of adults aged 18–24 years. Random sampling was done to select participants through computer generated process. Inclusion criteria were students aged 18–24 years, registered currently in university, having no diagnosed mental illnesses. Participants with diagnosed medicinal or psychiatric conditions were excluded. Data collection tools were TFEQ, IPAQ and GAD validated questionnaires and Anthropometric Parameters. IBM SPSS-21 was used for data analysis, and $p \leq 0.05$ was taken as statistically significant. **Results:** Out of 385 participants, females were 192 (49.87), while males were 193 (50.13%). The association between BMI and IPAQ status was not significant ($p=0.554$). An independent sample *t*-test revealed that there was no statistical significant association of cognitive restraint scores and uncontrolled eating scores with IPAQ status, where *p* were 0.875 and 0.403 respectively. **Conclusion:** Three-Factor Eating domains had no statistical significant relationship with physical activity and anxiety among university students despite the fact that degree of physical activity in this population has little bearing on compulsive eating behaviours.

Keywords: Anxiety, IPAQ, Students, TFEQ-R18

Pak J Physiol 2026;22(1):16–9, DOI: <https://doi.org/10.69656/pjp.v22i1.1881>

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INTRODUCTION

Obesity is body adiposity or an excess body fat.¹ Body mass index (BMI) has served as an extremely useful estimate of individual's weight relative to height since many years.² It is calculated as person's body weight (Kg) divided by square of height (m²).³ It is a commonly understood measure and easily calculated among researchers, general public, and clinicians, having a high specificity and low sensitivity.⁴

Anxiety disorders affect around 34% adults during their life-time and are related to significant impairment and distress, whereas prevalence for generalized anxiety disorder is 6.2%.⁵ A 7-item Generalized Anxiety Disorder scale (GAD-7) is used as brief screening tool for detection of anxiety.⁶ Better understanding of psychology of eating, including emotional, behavioural and cognitive aspects require more attention due to surging prevalence of obesity globally.⁷ Self-assessment questionnaires are of utmost importance for an evaluation of the eating behaviours. Presently, there are numerous questionnaires used widely for studying eating behaviour, amongst which Dutch Eating Behaviour Questionnaire, Three-Factor

Eating Questionnaire (TFEQ), and Restraint Scale, are most prominently cited.⁸ Usually eating disorders start in early years of teens, their estimated prevalence among college students was eleven-seventeen percent for females and four percent for males. Eating behaviour is broadly recognized as a result of internalized multi-dimensional constructs that encompass emotional, behavioural, and cognitive elements.⁹

In order to evaluate physical activity among the community, an international Physical Activity Questionnaire (IPAQ) was created. The shortened form of IPAQ has been evaluated extensively and is currently being utilized in several international investigations.¹⁰ Physical activity was categorized using an IPAQ scoring protocol. In 1998, WHO developed international Physical Activity Questionnaire–short form (IPAQ-SF), which is a common measurement tool for physical activity with seven questions.¹¹

The transition to higher education can significantly impact students' physical and mental health. Given the limited research on relationship between generalized anxiety disorder (GAD) and TFEQ with physical activity levels among young adults, a study investigating this association is warranted to

inform interventions and support services for this population. The objective of this study was to explore the relationship of eating domains with anxiety and physical activity among university students of Lahore.

METHODOLOGY

This cross-sectional study was conducted from April to October 2024 in University of Veterinary and Animal Sciences, and University of Engineering and Technology, Lahore. The study populace comprised of adults aged 18–24 years. Random sampling was done to select participants through computer generated process.

Students aged 18–24 years, registered currently in university, having no diagnosed mental illnesses were included, and those with diagnosed medical or psychiatric conditions were excluded.

Data collection tools were questionnaires and anthropometric parameters were weight (Kg), height (m), and BMI (Kg/m²). Sample size was calculated as 385 using the formula: $n = (Z\alpha/2)^2 [P(1-P)]/d^2$, where n is sample size, P is proportion (50%), $(Z\alpha/2) = 1.96$ and Z is confidence level (95%).

Our study employed 3 validated questionnaires, each targeting different aspects of this work:

- Three Factor Eating Questionnaire-R18 (TFEQ-R18): It is extensively used and recognized tool offering intuitions into how anxiety might influence eating patterns, together with responses to stress and emotional triggers. This questionnaire evaluates 03 dimensions of an eating behaviour: self-restraint, uncontrolled (binge) eating, and emotional eating.^{7,12}
- International Physical Activity Questionnaire (IPAQ): It helps in evaluating an amount of the physical activity performed by persons on weekly basis & provides a complete view of activity patterns. It designed to measure physical activity level across various intensities, including low, moderate, and vigorous activities.^{10,13}
- Generalized Anxiety Disorder-7 (GAD-7): It is made-up of seven items and is relatively accurate screening tool for identifying anxiety disorders and symptoms with great validity and reliability.⁶

IBM SPSS-21 was used for data analysis. Descriptive statistics were presented as frequencies, means and percentages. Chi-square test, independent sample t -test and one-way ANOVA were used to check associations of anxiety scores with physical activity patterns and eating behaviours, and $p < 0.05$ was taken as statistically significant.

RESULTS

Out of 385 participants, females were 192 (49.87%), while men were 193 (50.13%). Almost half (48.05%) of the students had normal BMI, 19 (4.94%) were underweight, 40.78% were overweight whereas (6.23%) belonged to obese category.

Severity of GAD symptoms was used to categorize these participants. Majority (264, 68.57%) individuals were in ‘Minimal’ anxiety category. One-fifth (20.78%), experienced ‘Mild’ anxiety, 31 (8.05%) were under ‘Moderate’ anxiety category, and 2.6% were in category of ‘Severe’ anxiety.

IPAQ status is categorized into two levels: moderate and low physical activity. The p -value for the association between gender and IPAQ status was 0.698, suggesting that there was no statistically significant association between gender and IPAQ status in this population. The table also examines the association between BMI categories and IPAQ status. Notably, in the obese category, none of the participants (0%) are classified as having moderate physical activity, while all 24 (100%) are categorized as having low physical activity levels. The p -value for the association between BMI and IPAQ status is 0.554, indicating that there is no statistically significant association between BMI and IPAQ status. (Table-1).

In particular, the mean scores for those with mild GAD were 1.87 ± 0.38 , for those with moderate GAD they were 1.85 ± 0.38 , for those with moderate GAD they were 2.00 ± 0.51 , and for those with severe GAD they were 1.93 ± 0.47 . One-way ANOVA was applied and $p = 0.882$ indicates that there is no statistically significant difference in cognitive restraint scores between the various GAD statuses, suggesting that the degree of cognitive restraint in these young adults’ eating behaviours is not significantly influenced by the severity of their GAD. Those with more severe GAD had higher scores on the Uncontrolled Eating Score, which gauges the propensity to overeat in response to outside cues. These variations are not statistically significant ($p = 0.483$). This shows that although there is a discernible trend of higher uncontrolled eating scores as GAD severity increases, the differences are not great enough to support a meaningful association between uncontrolled eating behaviour and GAD status. The degree to which people eat in reaction to their emotions is measured by the Emotional Eating Score, which varies amongst GAD statuses. The data indicates that there is no statistically significant difference in emotional eating scores between the various GAD categories ($p = 0.161$). (Table-2).

An independent sample t -test revealed that there was no statistical significant association of cognitive restraint scores and uncontrolled eating scores with IPAQ status, ($p = 0.875$ and 0.403 respectively). The degree to which eating is a reaction to emotions, measured by the Emotional Eating Score, was less in people with moderate physical activity (1.66 ± 0.5) than in people with low physical activity (1.97 ± 0.59), the difference is not statistically significant despite this variation ($p = 0.161$). This suggests that although there may be a tendency for people with lower levels of

physical activity to eat more emotionally, there is not a significant enough correlation between physical activity and emotional eating to warrant statistical analysis. (Table-3).

Table-1: Association of gender and BMI with international physical activity status in young adults

	IPAQ Status [n (%)]		<i>p</i>
	Moderate	Low	
Gender			
Male	3 (1.55)	190 (98.45)	0.698
Female	4 (2.08)	188 (97.92)	
BMI			
Underweight	1 (5.26)	18 (94.74)	0.554
Normal	4 (2.16)	181 (97.84)	
Overweight	2 (1.27)	155 (98.73)	
Obese	0 (0)	24 (100)	

Table-2: Relationship of 3-factor eating scores and anxiety status (GAD) among young adults studying in different universities of Lahore (Mean±SD)

TFEQ Scores	GAD Status				p
	Minimal	Mild	Moderate	Severe	
Cognitive Restraint Score	1.87±0.38	1.85±0.38	2±0.51	1.93±0.47	0.882
Uncontrolled Eating Score	1.94±0.34	2.14±0.46	2.4±0.45	2.04±0.54	0.483
Emotional Eating Score	1.91±0.51	2.08±0.7	2.19±0.69	2.06±1	0.161

One-way ANOVA applied

Table-3: Relationship of three-factor eating scores and international physical activity status (IPAQ) among young adults studying in different universities of Lahore (Mean±SD)

TFEQ	IPAQ Status		p
	Moderate	Low	
Cognitive Restraint Score	1.9±0.36	1.88±0.4	0.875
Uncontrolled Eating Score	2.13±0.31	2.02±0.4	0.403
Emotional Eating Score	1.66±0.5	1.97±0.59	0.161

Independent sample t-test applied

DISCUSSION

Physical activity is defined by WHO as ‘any bodily movement produced by the skeletal muscles which needs energy expenditure’, and it is ‘a substantial measure for improving health (both mental and physical)’.¹⁴

Globally depression and anxiety are solemn disabling conditions, correlating mental health illnesses with higher percentage of suicidal risks.^{15,16} Addressing the mental health matters of college students is essential as it's a latest concern amongst community and programmatic efforts to help students and promote intervention/prevention strategies.^{17,18}

There was no statistically significant association between gender and IPAQ status in this population. This is contrary to another finding¹⁹ where, physical activity levels exhibited a significant relationship with a gender ($p<0.001$). In our study there was no statistically significant association between BMI

and IPAQ status. This result implies that the BMI category does not significantly influence whether an individual is likely to engage in moderate or low physical activity within this group of young adults. This is contrary to another study¹⁹. Whereas, this is same as other research²⁰ where there was no significant correlation between BMI and physical activity.

Among children and adolescents, sedentary behaviour is vital health issue and is amplified with age.²¹ University students frequently experience a sitting time of more than 9 hours daily, which may trigger the onset of obesity. During adolescence, there is a notable decline in the amount of physical activity.^{19,22} Socio-economic status shows a crucial part in determining health consequences, including physical as well as psychological health.²³⁻²⁵ When it comes to treatment, many individuals in low income countries face significant obstacles to access treatment owing to financial constraints.^{26,27}

In this study, there was no statistically significant difference in cognitive restraint scores between various GAD statuses, suggesting that the degree of cognitive restraint in these young adults' eating behaviours is not significantly influenced by the severity of their GAD. This is not in line with another research²⁸. The emotional component of eating is not substantially correlated with the severity of GAD in this population, despite the fact that there is a slight increase in emotional eating scores with higher GAD severity. This is not in accordance to another research²⁸.

Emotional cues for instance loneliness, stress, and sadness have been reported previously as robust triggers for an over-eating, particularly in populaces with restricted emotional regulation strategies.²⁹ In the current study, there was no statistical significant association of cognitive restraint scores and uncontrolled eating scores with IPAQ status. This suggests that these young adults' degree of cognitive restraint in eating is not significantly influenced by their level of physical activity. This implies that the degree of physical activity in this population has little bearing on compulsive eating behaviours as well. This is in accordance to another research³⁰ where Three-Factor Eating domains showed insignificant relationship with physical activity levels.

LIMITATIONS

Short duration and small sample size were the main limitations of this study.

CONCLUSION

Three-Factor Eating domains had no statistical significant relationship with physical activity and anxiety among university students, despite the fact that degree of physical activity in this population has little bearing on compulsive eating behaviours.

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Received: 19 Jul 2025

Reviewed: 7 Sep 2025

Accepted: 7 Sep 2025

Contribution of Authors:

Authors approved the draft and are accountable in ensuring that questions related to accuracy or integrity of the work are duly investigated and resolved.

MHA: Wrote introduction and data collection

MAA: Wrote discussion

SIA: Wrote methodology

SB: Data analysis and results

MY: Data collection

SPS: Drafting of script

AM: Wrote discussion

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Conflict of Interest: None to declare

Funding: None received



ORIGINAL ARTICLE

POSTPARTUM DEPRESSION AT SECONDARY HEALTHCARE LEVEL
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Background: Postpartum depression is a global phenomenon. The magnitude is even wider when it comes to developing countries compared to industrialized counterparts. Identification of depression by healthcare works at levels is necessary to provide adequate support and resources to women suffering from postpartum depression to improve their mental health and overall wellbeing. This study aimed to determine the factors associated with depression among postpartum patients presenting to a secondary healthcare facility in Timergara District, Lower Dir. **Methods:** A total of 382 women, 6 to 8 weeks postpartum were registered from 1st March, 2024 to 31st October, 2024. Participants' and their partners' sociodemographic profile as well as obstetrical, neonatal, and psychiatric data were gathered using a self-devised structured questionnaire. DSM-V criteria were used to identify postpartum depression disorder. SPSS-25 was used to analyze data. **Results:** Fifty-seven (57.2%) patients were in the age group 18–30 years, 98 (25.65%) patients fulfilled the definition of postpartum depression. Their mean age was 29.07±5.59 years. A significant association ($p<0.05$) was observed between the planned nature of pregnancy, infant's gender and the patient's relation with in-laws with postpartum depression. **Conclusion:** Postpartum depression is prevalent among younger mothers particularly those experiencing social dissatisfaction, unplanned pregnancies and giving birth to female babies.

Keywords: Depression, DSM-V, Pakistan, Postpartum, Resource-limited settings

Pak J Physiol 2026;22(1):20–3, DOI: <https://doi.org/10.69656/pjp.v22i1.1806>

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INTRODUCTION

Postpartum period is a high-risk period when women are prone to many mental health conditions like postpartum blue, depression and even psychosis. Women may have postpartum depression (PPD) as early as one month after giving birth.¹ A meta-analysis and thorough assessment of PPD revealed that 24.7% of postnatal women suffer from mental diseases worldwide.² Given the potential impact of maternal mental health conditions on the mother's physical wellbeing, child development, and family and societal relationships, PPD is recognized as a public health concern.³

The mental wellbeing of mothers can have implications for the development of attachment insecurity in their offspring, as well as the mental health of adolescents.⁴ Children with negligent parenthood as a consequence of compromised maternal mental health are at risk of becoming negligent parents themselves leading to a perpetuated cycle of dysfunctional family dynamics. Children of depressive disorders women are more prone to restricted development, diarrhoeal episodes, and infectious diseases.^{4,5}

Multiple studies have revealed that occupation, educational level, being primigravida, gender of the newborn, prior episodes of depression (including amid gestation), companion's depressive disorders, discontentment with the spouse, and an absence of interpersonal assistance were among the key variables

that lead to depressive symptoms in women following delivery.^{6,7} In another study BMI, mode of delivery, family type, number of children alive, exclusive breastfeeding, and frequency of physical activity had an association with PPD.⁸ The financial status of families, which is directly proportional to access to medical care and education, have been argued in some research to be more significant than these other determinants.⁹

Considering infant gender, some studies have observed a higher risk of PPD among mothers of girls, while some contradict that a significant association exists.^{10,11} Some findings from earlier research point to a possible increase in PPD incidence among women residing in metropolitan settings.⁹ Prevalence of PPD was found to be 30%¹² in Pakistan, 17% in developing countries, and 11% in developed countries.¹³

The aim of this study was to determine the frequency of depression in postpartum patients and factors associated with it in a secondary healthcare facility in Lower Dir area of Pakistan. This study will help to create awareness among gynaecologists and obstetricians to pay attention to mental health of patients prone to PPD.

MATERIAL AND METHODS

This cross-sectional study was conducted, after approval from Ethical Review Board of Saidu Medical College, Swat, at the Department of Obstetrics and Gynaecology, at a secondary level hospital in Timergara Tehsil,

District Lower Dir of northern Pakistan. Our estimated sample size was 382 at 95% confidence level, anticipated frequency of 46%¹⁴ and 5% margin of error. All women who attended the hospital at 6–8 weeks postpartum were included in the study. A self-devised structured questionnaire was used to collect data after getting written informed consent from the patients. Women who had given birth to twins or had any comorbidities like thyroid problem, hypertension, diabetes or any respiratory or cardiac issues were excluded.

Six components of a structured questionnaire were used to collect data *viz.* socio-demographic data, obstetric and child information, marital life such as the quality of relationships as well as availability of assistance from families, information on parenting, and questions on participants' and partners' past histories of depression. Both prenatal care logbooks and interviews were used to gather information on demographics, obstetrics, infants, and social support. Information about all these factors was recorded and its association with postnatal depression was studied. Postpartum Depression was diagnosed using DSM-V criteria, defined as presence of ≥ 5 of symptoms namely: Depressed mood, disturbed appetite, disturbed sleep (insomnia or hypersomnia), lack of interest in activities or pleasure (anhedonia), physical agitation or retardation, fatigue or loss of energy, feeling of being worthless or excessive guilt, decreased concentration, and recurrent thoughts of death.¹⁵

Data analysis was done using SPSS-25. Variables were sorted into four primary groups for statistical analysis: demographics, obstetric and newborn features, information about marital relationships, and couple's histories of depression. Continuous variables like patient's age, income, and partner's age was tabulated as Mean $\pm$ SD. Categorical variables like gender of infant, patient's education, occupation, residence, parity and mode of delivery were described in terms of frequencies and percentages. Postpartum depression was stratified against patient's demographic and obstetric profile, factors related to infant, partner, social or family circumstances and previous history of depression. Association was determined by applying Chi-square test and $p \leq 0.05$ was considered statistically significant.

RESULTS

This study included a total of 382 patients. The age of the patients ranged from 18 to 40 years with mean age 29.07 $\pm$ 5.59 years. Among them, 98 (25.65%) patients satisfied the DSM-V criteria for PPD. Among those 98 patients who had postpartum depression, most of the patients belonged to the age group 18 to 30 years, comprising 57 (58.1%) participants. The mean duration of marriage of the patients was 5.680 $\pm$ 1.030 years. Among the PPD patients 61 (62.2%) had none or

limited schooling, 21 (21.4%) participants attained high school and 16 (16.3%) participants had higher education. A major portion (73, 74.5%) of the participants were housewives/unemployed, 51 (52.0%) participants had family income ranging Rs. 50,000 to 100,000 per month. The distribution of rural and urban residence was 58 (59.2%) vs 40 (40.8%) respectively.

Fifty-three (54.1%) patients having PPD were first time mothers. Sixty-five (66.3%) patients had vaginal delivery with or without instrumentation. The mean gestational age was 38.028 $\pm$ 0.879 weeks. Fifty-eight (59.3%) patients had female babies. (Table-1).

Sixty-one (62.2%) patients did not receive the expected social support from their in-laws and spouse. The majority 67 (68.4%) of PPD patients were living in a combined family system. Seventeen (17.3%) patients had a previous history of depression. Partners of 9 (9.2%) patients had a history of depression. (Table-2).

Table-1: Association of obstetric and infant factors with PPD (n=382) [n (%)]

	PPD		Total	χ^2	p
	Yes (n=98)	No (n=284)			
Gestational Age					
37–38 weeks	69 (70.4)	178 (62.7)	247	1.868	0.171
39–40 weeks	29 (29.6)	106 (37.3)	135		
Parity					
Primi-parity	53 (54.1)	126 (44.7)	179	2.723	0.098
Multi-parity	45 (45.9)	158 (55.6)	203		
Nature of pregnancy					
Planned	82 (83.7)	154 (54.2)	236	26.752	<0.001*
Unplanned	16 (16.3)	130 (45.8)	146		
Delivery					
NVD	65 (66.3)	186 (65.5)	251	0.022	0.882
C-Section	33 (33.7)	98 (34.5)	131		
Infant's Gender					
Male	40 (40.8)	189 (66.5)	229	13.026	<0.001*
Female	58 (59.2)	95 (33.5)	153		

*Statistically significant

Table-2: Association of family circumstances/social and partner factors with PPD (n=382) [n (%)]

	PPD		Total	χ^2	p
	Yes (n=98)	No (n=284)			
Relation with in-laws/partner					
Satisfied	37 (37.8)	210 (73.9)	247	42.305	<0.001
Unsatisfied	61 (62.2)	74 (26.1)	135		
Family system					
Joint	67 (68.4)	214 (75.4)	281	1.795	0.180
Nuclear	31 (31.6)	70 (24.6)	101		

*Statistically significant

DISCUSSION

This study was conducted in a far-flung area of Pakistan with limited healthcare facilities. It was observed that 25.65% of women seeking postpartum treatment at our facility exhibited symptoms suggestive of PPD. The range of PPD prevalence is around 17% in developing countries, whereas in developed countries, the figure drops to around 11%.¹³ Results from our study show a higher prevalence as compared to the average for developing countries. In Pakistan, the prevalence of

postpartum depression has been estimated to be around 30%.¹² Our findings are in agreement to the local study. This is expected as the study was conducted in a region with very strong cultural hierarchies.

It has been observed that self-reported symptoms serve as a reliable predictor of the proportion of postpartum mental problems.^{14,16} The responses to questions pertaining to self-reported symptoms, asked at the secondary health facility during this study, were used to diagnose probable PPD. There is a high possibility of prevalence of PPD being greater than our observations when determined by psychiatrists, and more refined tools are used. However, this simple approach to the diagnosis of PPD can serve as the first step in the referral process so that no woman needing therapy is neglected.

Low contentment with the gender of the baby and prenatal mood disorder were factors linked to probable PPD in previous studies.¹⁷ Our study also shows similar findings with female gender of the baby and previous history of depression showing significant association with PPD. This highlights the deep impact of societal norms preferring a male child over a female on the mother despite having no control over the factor.

Unintended pregnancy has also been linked to PPD.¹⁸ According to previous prospective research, unexpected pregnancies increase the risk of postpartum depression (PPD) and parenting stress in women by causing marital conflict and poor involvement of father in child care.¹⁹ A multi-country study also discovered that unplanned pregnancy was associated with an elevated risk of developing depression during the postnatal period.²⁰ Although the effect of unexpected pregnancy on PPD peaked at 4 months after delivery, the effect on parenting stress persisted for up to 2 years, suggesting long-term repercussions on mother and child health.²¹ Other than unplanned nature of pregnancy, primi gravidity and a history of miscarriage is shown to be associated with increased risk of developing depression in the postnatal period.²² Our study suggests a similar association between planned nature of a pregnancy and presence of PPD.

The majority of research has consistently suggested a strong relationship between PPD and poor birth experiences. According to a previous cohort study, PPD developing as a result of post-traumatic stress disorder has been shown to have a detrimental impact on interpersonal relationships in couples.²³ Satisfaction with the birth experience is mainly impacted by women's prenatal expectations, but it also includes several other elements including protection, encouragement, admiration, confidentiality, and involvement in family planning decisions.²⁴ Birth experience is also affected by differences in home and institutional deliveries.²⁵ An indirect impact of pre-partum depression scores and birth experience on

postpartum maternal-child bonding, which is mediated by postpartum depression, was also described by Eitenmüller *et al*.²⁶

Previous studies have consistently indicated that a woman's relationship with her spouse significantly influences her mental wellbeing. PPD, unwanted pregnancy, birth experience and couple relationships, all have an impact on one another.^{25,26} Couples are nonetheless susceptible to psychological issues and marital discontent since the move stresses out their life and accentuates their differences.²⁷ Research was conducted among Indonesian mothers to explore the extent to which PPD is affected by spousal relationships, husband involvement, and maternal health behaviour revealing that negative interactions throughout gestation and postpartum for both parents.²⁸ To support parents' mental health, healthcare institutions may be able to offer classes on birthing education and enhance the dynamics of co-parenting for couples.²⁹ Jamshaid *et al*.³⁰ have suggested that PPD acts as a mediator between attachment trauma and maternal self-efficacy, whereas emotional support is a moderator.

This study was conducted in a setting not usually considered for screening or treatment of psychiatric disorders ignoring an important component of maternal and child health which has a substantial effect on family dynamics. Hence it has some limitations. The use of a previously validated questionnaire might have strengthened the study but due to a lack of trained personnel for data collection and the simplicity of purpose, DSM-V criteria have been used. The scope of this study was limited only to exclude presence or absence of PPD and did not consider its severity. Future studies with qualitative components integrated into the study might be able to give better insights into the problem in our particular settings.

CONCLUSION

Frequency of postpartum depression in our study population was 25.65%, and majority of the patients were younger mothers aged 18–30 years. Significant association of postpartum depression was found with obstetric, social, and familial factors, and primiparous mothers were affected more than multiparous mothers. Unplanned pregnancy and female babies were significantly associated with PPD.

RECOMMENDATIONS

Development of depression should be kept in mind while taking care of the mother during early postpartum period. Prevention and treatment of PPD should be tailored to the individual needs of each mother and may require a multidisciplinary approach.

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Received: 25 Dec 2024

Reviewed: 3 Nov 2025

Accepted: 9 Nov 2025

Contribution of Authors:

Authors approved the draft and are accountable in ensuring that questions related to accuracy or integrity of the work are duly investigated and resolved.

NW: Study design, Data collection, Manuscript writing

ZN: Data analysis, manuscript writing and references literature

SA: Data entry, data analysis, literature search and manuscript writing

HI: Literature search and reference writing

KM: Proof reading, Literature search, reference writing

AB: Literature search, reference writing

Conflict of Interest: No conflict of interest is declared, **Funding:** No funds have been received from any agency



ORIGINAL ARTICLE

EFFICACY OF ORAL TRANEXAMIC ACID VERSUS PLACEBO IN THE TREATMENT OF EPIDERMAL MELASMA

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Background: Epidermal melasma, a common hyperpigmentation disorder, can cause significant psychological and social distress. This study aimed to evaluate the efficacy of oral tranexamic acid (TXA) in treatment of epidermal melasma. **Methods:** This cross-sectional study was conducted at the Dermatology Department, PIMS, Islamabad, from Oct 2020 to Apr 2021. A total of 202 patients aged 13–60 years with epidermal melasma for at least six months, and a Melasma Area and Severity Index (MASI) score of 20–36, were randomly assigned to two groups. Group A received oral TXA (250 mg twice daily), while Group B received a placebo. Patients were assessed at four-week intervals, with final evaluation at 12 weeks. Statistical comparisons were performed using *t*-tests and Chi-square tests. **Results:** At three months, the mean MASI reduction was significantly greater in Group A (22.6±8.1) than in Group B (13.7±6.6, $p=0.001$), with a higher mean percent reduction in Group A (60.2±20.3 vs 37.7±19.5%), ($p=0.001$). An excellent or good response was observed in 67.3% of Group A patients compared to 21.8% in Group B ($p=0.001$). **Conclusion:** Oral TXA significantly reduced MASI scores compared to placebo, demonstrating superior efficacy in improving epidermal melasma.

Keywords: Efficacy, Epidermal Melasma, MASI score, Placebo, Tranexamic Acid, Treatment

Pak J Physiol 2026;22(1):24–7, DOI: <https://doi.org/10.69656/pjp.v22i1.1841>
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INTRODUCTION

Melasma is a frequently encountered disorder characterised by the development of pigmentation on the face. It tends to appear in a symmetrical pattern and is more commonly observed in females and individuals with darker skin tones.^{1,2} The condition is distinguished by irregular dark brown spots and patches, primarily appearing on parts of the skin that are exposed to sunlight, particularly the face.³ The exact cause of this condition is not fully comprehended, however, it is widely acknowledged that hyperactive melanocytes, stimulated by ultraviolet light, are the primary factor. Additional considerations encompass genetic predisposition, hormone-based treatment, photo-sensitizing medications, and the state of being pregnant.⁴ Melasma is categorised as dermal, epidermal, or mixed types, according to the level at which the pigment is located. Given its aesthetically unappealing appearance, melasma can lead to substantial psychological and social difficulties, emphasising importance of therapy.^{5,6}

The standard treatment usually consists of using a sunscreen that protects against a wide range of ultraviolet rays, along with depigmenting substances such as hydroquinone, azelaic acid, retinoic acid, kojic acid, and different natural plant extracts including licorice root and mulberry extracts. Additional therapeutic options encompass chemical peels and laser treatments, which have the potential to induce irritation, post-inflammatory hyperpigmentation, contact

dermatitis, or incur significant costs.^{7,8} Tranexamic acid has been identified as a promising remedy for melasma. While the precise process is not yet understood, it is believed that it inhibits the interaction between melanocytes and keratinocytes, and has the potential to reverse the dermal alterations linked to enlarged blood vessels in melasma. Tranexamic acid is generally well tolerated with minimal side effects. However, at dosages over 500 mg/day, the adverse effects can become more noticeable.⁶ These side effects may include oligomenorrhoea, deep vein thrombosis, and pulmonary embolism. It is important to note that no thromboembolic consequences have been documented with the modest doses of tranexamic acid used for treating melasma.⁹

While existing treatments can cause irritation and are often costly, tranexamic acid offers a potentially effective and well-tolerated alternative. This study aims to evaluate the efficacy of oral tranexamic acid versus placebo in treating epidermal melasma, providing new insights into its therapeutic potential and safety profile. By comparing it directly to a placebo, this research will add robust evidence to support its use and guide future clinical practices.

MATERIAL AND METHODS

With ethical committee approval (F.2-11/SZABMU/AS&RB-52/2018), the comparative cross-sectional study was conducted at the Dermatology OPD of PIMS,

Islamabad. After obtaining written informed consent, the size and severity of each melasma lesion were recorded.

The sample size was calculated using the WHO Sample Size Calculator based on the following parameters: a level of significance (α) of 5%, a power of test ($1-\beta$) of 90%, an anticipated efficacy of tranexamic acid (P1) of 0.49, and an anticipated efficacy of placebo (P2) of 0.27. This resulted in a total of 202 patients being enrolled in the study, with 101 patients in each group.¹⁰ Inclusion criteria included patients of either gender, aged between 13 and 60 years, with a diagnosis of epidermal melasma that had been present for six months or more, and a baseline MASI score of 20–36. Exclusion criteria encompassed patients who had been using other melasma therapies within the past two months, pregnant or lactating patients, those taking oral contraceptive pills, individuals with a history of any endocrine disorder, patients with thromboembolic disorders, and those with a deranged clotting profile and PT/INR.

Patients were assigned to Group A (oral tranexamic acid capsule 250 mg BD) or Group B (similar-looking placebo capsule) using the lottery method. Complete blood profile and clotting profile were checked initially and monthly thereafter. Patients were instructed to apply broad-spectrum standardized sunscreens and discontinue any other therapies during the treatment period. Follow-ups were scheduled every 4 weeks to analyse changes in melasma, grading responses as excellent, good, fair, or poor. Final analysis was conducted after 12 weeks, with treatment considered efficacious if the response was excellent, good, or fair, and not effective if the response was poor.

Data were entered and analysed using SPSS-24. Mean and standard deviation were calculated for quantitative variables (age, MASI score at baseline and after treatment). Frequencies and percentages were calculated for qualitative variables (gender, efficacy). Efficacy between the tranexamic acid and placebo groups was compared using Chi-square test, with a $p \leq 0.05$ considered statistically significant.

RESULTS

There were 24 (23.8%) males and 77 (76.2%) females in the tranexamic acid (TXA) group, while the placebo group had 18 (17.8%) males and 83 (82.2%) females. Regarding age distribution, 77 (76.2%) patients in the TXA group and 90 (89.1%) patients in placebo group were aged between 19–40 years. In the 41–60 years age group, there were 24 (23.8%) patients in the TXA group and 11 (10.9%) patients in the placebo group. The mean age was 35.1 ± 8.7 years for the TXA group and 33.7 ± 5.1 years for the placebo group, with an overall mean age of 34.4 ± 7.3 years. (Table-1).

In both groups, the mean MASI score at baseline was 37.9 ± 6.1 for the tranexamic acid (TXA) group and 37.1 ± 4.9 for the placebo group, with no

significant difference between them ($p=0.354$). However, at months 1, 2, and 3, the MASI scores were significantly lower in the TXA group compared to the placebo group ($p < 0.001$ for all). The mean change from baseline at 3 months was higher in the TXA group (22.6 ± 8.1) than in the placebo group (13.7 ± 6.6), with a significantly greater percent change from baseline in the TXA group (60.2 ± 20.3) compared to the placebo group (37.7 ± 19.5) ($p < 0.001$ for both). (Table-2).

Response to therapy at 3 months differed significantly between the groups. In the tranexamic acid (TXA) group, 30.7% of patients had an excellent response, 36.6% had a good response, 26.7% had a fair response, and 5.9% had a poor response. Conversely, in the placebo group, only 9.9% had an excellent response, 11.9% had a good response, 48.5% had a fair response, and 29.7% had a poor response ($p=0.001$). (Table-3).

At 3 months, efficacy of treatment differed significantly between the groups. In the tranexamic acid (TXA) group, 67.3% of patients showed efficacy, whereas in the placebo group, only 21.8% showed efficacy ($p=0.001$). (Table-4).

At 3 months, efficacy of treatment stratified by gender showed significant differences between the groups. In males, 66.7% of those in the tranexamic acid (TXA) group and 33.3% in the placebo group showed efficacy ($p=0.032$). Similarly, in females 67.5% in the TXA group and 19.3% in the placebo group showed efficacy ($p=0.001$). (Table-5).

Table-1: Gender and age distribution in groups

Variables	TXA	Placebo	Total
Males	24 (23.8%)	18 (17.8%)	42 (20.8%)
Females	77 (76.2%)	83 (82.2%)	160 (79.2%)
19–40 Years	77 (76.2%)	90 (89.1%)	167 (82.7%)
41–60 Years	24 (23.8%)	11 (10.9%)	35 (17.3%)
Mean$\pm$SD Age (Years)	35.1 ± 8.7	33.7 ± 5.1	34.4 ± 7.3

Table-2: MASI score at different times and change from baseline in both groups (t-test)

MASI Score Assessment	TXA	Placebo	<i>p</i>
Baseline	37.9 ± 6.1	37.1 ± 4.9	0.354
Month 1	30.1 ± 5.8	33.2 ± 4.9	< 0.001
Month 2	23.8 ± 6.7	28.2 ± 6.4	< 0.001
Month 3	15.2 ± 8.4	23.4 ± 8.4	< 0.001
Change from Baseline at 3 Months	22.6 ± 8.1	13.7 ± 6.6	< 0.001
Percent Change from Baseline at 3 Months	60.2 ± 20.3	37.7 ± 19.5	< 0.001

Table-3: Response to therapy at 3 months in both groups (Chi-square) [n (%)]

Response Category	TXA	Placebo	Total	<i>p</i>
Excellent	31 (30.7)	10 (9.9)	41 (20.3)	< 0.001
Good	37 (36.6)	12 (11.9)	49 (24.3)	
Fair	27 (26.7)	49 (48.5)	76 (37.6)	
Poor	6 (5.9)	30 (29.7)	36 (17.8)	

Table-4: Efficacy at 3 months in groups [n (%)]

Efficacy	TXA	Placebo	Total	<i>p</i>
Present	68 (67.3)	22 (21.8)	90 (44.6)	0.001
Absent	33 (32.7)	79 (78.2)	112 (55.4)	

Table-5: Efficacy at 3 months in both groups (stratification of gender) [n (%)]

Gender	Efficacy	TXA	Placebo	Total	p
Males	Present	16 (66.7)	6 (33.3)	22 (52.4)	0.032
	Absent	8 (33.3)	12 (66.7)	20 (47.6)	
Females	Present	52 (67.5)	16 (19.3)	68 (42.5)	0.001
	Absent	25 (32.5)	67 (80.7)	92 (57.5)	

DISCUSSION

Epidermal melasma presents a therapeutic challenge due to its chronic and recurrent nature. While various treatments exist, their efficacy and tolerability remain variable. This study investigates the efficacy of oral tranexamic acid versus placebo in managing epidermal melasma, aiming to provide evidence-based guidance for clinical practice. Tranexamic acid depigments via inhibiting plasmin activator. Preventing plasminogen binding to keratinocytes reduces free arachidonic acid, prostaglandins, melanocyte tyrosinase activity, and melanocyte-stimulating hormone. VEGF and endothelin-1 are also decreased. Besides this, tranexamic acid fights skin problems by reducing inflammation and allergies.^{11,12}

Our study results are similar to Del Rosario E *et al*¹⁰, who determined the efficacy of oral Tranexamic acid in patients with melasma of moderate to severe degree. Their results showed a 49% reduction in mMASI score in the TXA group versus 18% in the control group at 3 months of treatment. They further demonstrated that 3 months after treatment was stopped, there was a 26% reduction in mMASI score in the TXA group compared with the baseline visit versus a 19% reduction in the placebo group. Our study results are similar with an efficacy of 67.3% with TXA versus 21.8% in placebo group. We, however, did not monitor the patients further after 3 months.

Our study demonstrated a significantly higher mean change from baseline at 3 months in the TXA group (22.6±8.1) compared to the placebo group (13.7±6.6), with a notably greater percent change from baseline in the TXA group (60.2±20.3) versus the placebo group (37.7±19.5) ($p<0.001$ for both), consistent with Minni *et al*¹³, where significant reductions in MASI scores were observed within each treatment group. Sarwar *et al*¹⁴ demonstrated 40% reduction in melasma in patients on oral TXA supporting our findings.

Our study results corroborate with Batra *et al*¹⁵ who observed good results in 25% and very good results in 70% cases on oral TXA. Colferai *et al*¹⁶ demonstrated significant improvements in MASI scores, with the MELASQoL value increasing from 55.0 to 56.1 in their treatment group. Conversely, Del Rosario *et al*¹⁰ reported a 49% reduction in mMASI score in the tranexamic acid group at 3 months, while our study showed a significantly higher mean change from baseline at 3 months in the TXA group compared to the

placebo group, with a greater percent change. These findings collectively support the efficacy of oral tranexamic acid in improving melasma severity, highlighting its potential as a valuable therapeutic option for patients with this condition.

Our findings align with Mushtaq *et al*¹⁷ where efficacy was observed in 67.3% of patients in the tranexamic acid group compared to 21.8% in the placebo group, indicating a significant improvement in melasma severity. Minni *et al*¹³ reported a marked improvement in melasma with oral tranexamic acid treatment, with 65.6% of patients showing improvement compared to 27.1% in the placebo group after 12 weeks. Our study demonstrated a similar trend of efficacy with TXA treatment, showcasing its potential as a promising therapeutic option for melasma management. However, it's imperative to note the side effects observed, consistent with Minni *et al*¹³ indicating the importance of monitoring adverse events with TXA therapy.

One limitation of the study is the absence of long-term follow-up to assess the durability of treatment response. However, a notable strength lies in the utilization of a randomized controlled trial design coupled with comprehensive statistical analysis.

CONCLUSION

MASI scores declined significantly over 1, 2, and 3 months in both groups, with lower scores in the oral tranexamic acid group compared to placebo. The mean absolute and percent decrease in MASI scores at 3 months, as well as the efficacy (excellent or good response), were significantly better in the TXA group.

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Received: 3 Apr 2025

Reviewed: 17 Feb 2026

Accepted: 7 Mar 2026

Contribution of Authors:

Authors approved the draft and are accountable in ensuring that questions related to accuracy or integrity of the work are duly investigated and resolved.

JSR: Concept of study, data collection, Manuscript writing

SS: Data collection and manuscript writing

ME: Data collection and interpretation

SL: Data analysis and manuscript writing

HA: Data collection and interpretation

MT: Critical analysis of final manuscript

Conflict of Interest: No conflict of interest is declared

Funding: No funds have been received from any agency



ORIGINAL ARTICLE

AMELIORATIVE EFFECTS OF MELATONIN ON HISTOPATHOLOGICAL CHANGES IN LUNG TISSUE OF NICOTINE-EXPOSED RATS

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Background: Melatonin, a potent naturally occurring antioxidant plays a significant role in counteracting various toxins. This study aimed to evaluate the ameliorative effects of melatonin on histopathological changes in lung tissues of nicotine-exposed rats. **Methods:** This quasi-experimental study was conducted at Isra University, Hyderabad during Feb-May 2024. Forty Wistar albino rats were used. Rats were evenly divided into four groups. Group A (control group) received only normal chow, Group B received nicotine hydrogen bitartrate 0.6 mg/Kg bodyweight daily intra-peritoneal, Group C received same dose of nicotine with melatonin 5 mg/Kg I/P daily, and Group-D received the same dose of nicotine with melatonin 10 mg/Kg I/P. Serum inflammatory and antioxidant markers were analysed along with the histopathological analysis. **Results:** A significant rise in haematological parameters was observed in Group B compared to the control ($p<0.05$). Group D showed more prominent decline in these parameters ($p<0.05$) compared to Group C. A significant rise in c-reactive protein levels (from 0.10 ± 0.11 to 0.88 ± 0.25 mg/dL) and fibrinogen levels (from normal 224.7 ± 74 to 447.1 ± 0.41 mg/dL) were observed in Group B rats. The anti-oxidative and tissue peroxidative makers (MDA, SOD and GSH) levels were lower in Group C and D compared to control group, however, Group B showed a significant ($p<0.05$) decrease in these. **Conclusion:** Melatonin has a protective effect against inflammatory, oxidative and proliferative reactions resulting from nicotine to lung tissues.

Keywords: Anti-inflammatory, Anti-oxidative, Lung tissue, Melatonin, Nicotine

Pak J Physiol 2026;22(1):28–32, DOI: <https://doi.org/10.69656/pjp.v22i1.1883>

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INTRODUCTION

The lung is an organ composed of a complex network of tubes. It takes part in several vital physiological processes, such as gas exchange and immunological reactions. Respiratory infections are prevalent and represent a substantial danger to public health.¹ Over the past 10 years, there has been a rise in the number of lung disease-related deaths, particularly chronic obstructive pulmonary disease (COPD), which is brought on by the harmful effects of cigarette smoking.¹ Since cigarette smoke is directly inhaled into the lungs, it is one of the main risk factors for damage to multiple organs, particularly the lungs, which is the cause of death from cardiorespiratory failure.² Cigarette smoking causes one-third of all cancer-related deaths and ranks as a primary cause of lung cancer.³ Nicotine is one of the over 9,500 components of cigarette smoke. The primary pharmacologically active ingredient in tobacco smoke's particle phase and the highly poisonous molecule that causes the majority of harmful effects of smoking, is nicotine.⁴ By producing reactive oxygen free radicals, nicotine damages the lungs oxidatively. This frequently leads to a drop in the levels of glutathione peroxidase, catalase, and superoxide dismutase.⁵

The pineal gland produces the endogenous

hormone melatonin (N-acetyl-5-methoxytryptamine) primarily from the amino acid tryptophan. It shares structural similarities with serotonin and is secreted in the dark in both mammals and humans.⁶ Its exogenous dietary sources include melatonin-rich foods like orange juice, walnuts, and sour cherries.⁷ It has anti-inflammatory, anti-proliferative, anti-cancerous, anti-apoptotic (in injured cells), and pro-apoptotic (in malignant cells) qualities in addition to its potent antioxidant qualities. Even when administered before or concurrently with chemotherapy and radiation therapy, it has synergistic effects.^{8,9}

Melatonin effectively suppresses inflammatory mediators, including IL-6, TNF- α , and MMP-9, as well as inflammatory cells, to achieve its anti-inflammatory effects.¹⁰ Melatonin helps in preventing lung fibrosis brought on by cigarette smoke. This is achieved primarily by decreasing the production of TGF- β 1 and collagen 1 and concurrently increasing the production of antioxidants glutathione peroxidase and superoxide dismutase.¹¹

Being a naturally occurring hormone, melatonin is far safer to use both therapeutically and prophylactically than other medications. It is also widely accessible and has very few adverse effects. Research on humans and animals has demonstrated its safety for

short-term and long-term treatments.⁶

The primary objective of the present study was to evaluate the ameliorative effects of melatonin on histopathological changes in the lung tissue of nicotine-exposed rats.

MATERIAL AND METHODS

After obtaining approval from the Ethical Committee, this quasi-experimental animal study was conducted jointly by the Departments of Biochemistry and Pharmacology, Isra University, Hyderabad, from 5 Feb to 30 May 2024. Animal procedures were performed at Sindh Agriculture University, Tandojam, Hyderabad. Forty rats were selected through the non-probability consecutive sampling technique. The sample size was determined using power analysis.¹²

The procured Wistar albino rats were housed in separate cages with free access to food and water. The temperature and light/dark cycles were maintained at standard levels.¹³ The animals were divided randomly into 4 groups (A, B, C, and D), each comprising of 10 rats. For 4 weeks, Group A (Control group) was given regular saline. Group B (nicotine-exposed group) rats received daily nicotine injections at 0.5 mg/Kg through intraperitoneal administration while the rats experienced cigarette smoke exposure for 30 minutes every day inside a glass chamber. Group C received melatonin via oral administration at a dose of 5 mg/Kg daily, while the rats received nicotine at 0.5 mg/Kg through intraperitoneal injection and experienced cigarette smoke for 30 minutes each day. Group D received melatonin through oral administration at 10 mg/Kg daily dose, while the rats received nicotine at 0.5 mg/Kg through intraperitoneal injection and experienced cigarette smoke for 30 minutes each day.

At the end of the 4th week, all rats were kept fasting for 18 hours and sacrificed by decapitation. Fasting blood samples of 2 ml in EDTA-tube were collected. The blood samples from each rat were collected from the retrograde orbital plexus before the sacrifice in a capillary tube under anaesthesia. The collected blood samples were sent to the Isra University Diagnostic Laboratory for estimation of Red blood cells (RBCs), Haemoglobin (Hb), White blood cells (WBCs), and Platelets, along with C-reactive protein (CRP), Fibrinogen, Interleukin-6 (IL-6) and Tumour Necrosis Factor alpha (TNF- α). After employing commercially available kits obtained from BioAssay Technologies China, the analyses of Superoxide Dismutase (SOD) activity, Malondialdehyde (MDA), and Glutathione GSH levels were carried out.

Lung specimens of the rats for histological investigation were taken after dissection and preserved in 10% formalin. The slides for histological investigation were processed and stained.¹⁴ The distinctive histological alterations and findings of lung

samples were recorded on a pre-designed proforma. Data were entered and analysed on SPSS-25. The Mean $\pm$ SD of various parameters for each group were noted. ANOVA followed by post-hoc Tukey was applied, and $p \leq 0.05$ was considered statistically significant.

RESULTS

Table-1 presents the post-hoc Tukey test of haematological parameters in groups. Rats in Group B had significantly higher RBC counts, Hb concentrations, WBC count, and Platelets at the end of the experiment, compared to control group ($p < 0.05$). Group D rats showed a significant reduction in haematological parameters including RBCs, Hb concentration, WBC, and platelet count, compared to Group C.

In comparison to Group B, melatonin administration effectively decreased the serological inflammatory markers in Group C and D ($p < 0.05$). The levels of CRP, fibrinogen, IL-6 and TNF- α levels decreased in both treatment groups. However, these reductions were more pronounced in Group D than in Group C for all markers ($p < 0.05$) (Table-2).

Table-3 shows the oxidative stress marker levels (MDA, SOD, GSH) across all study groups. Group B showed significantly elevated MDA while depleting SOD and GSH compared to Group A ($p < 0.05$). A moderate-dose melatonin (Group C) provided intermediate MDA restoration, whereas high-dose (Group D) nearly normalized it. Moreover, the nicotine-induced reduction in SOD and GSH activity was significantly mitigated by the administration of melatonin in different doses.

Figure-1 depicts the histo-microphotographs of lung tissue sections of all study groups. Histo-pathological analysis of the lungs of Group A rats showed regular histological architecture for the bronchial tree and alveoli. However, the lung tissue of the animals in Group B showed notable histological changes, such as inter-alveolar septal hypertrophy (blue arrow) and proliferation, as well as mononuclear cell infiltration and fibrous growth. There was perivascular oedema, hyalinization patches (orange star), and a small amount of inflammatory cell infiltration in the blood vessels (yellow arrow). Group B rats also exhibited thickening of the interalveolar septa, bronchial epithelial necrosis, and desquamation. Group C showed good responses to all histopathology variables of detrimental effects of nicotine exposure such as increased cellularity and partially preserved alveolar spaces (green arrows), to the low dose of melatonin treatment. A more prominent damage protecting effect to the nicotine was observed after the administration of a 10 mg/Kg dose of melatonin in the treatment Group D (yellow arrow).

Table-1: Haematological parameters across study groups (Mean±SD)

Test Parameters	Groups				p
	A	B	C	D	
RBCs (million/mm ³)	4.47±0.29 ^B	5.28±0.22 ^A	4.93±0.20	4.72±0.43 ^B	0.004
Hb (g/dL)	8.28±0.21 ^{B,C,D}	10.26±0.32 ^{A,D}	9.87±0.03 ^{A,D}	9.01±0.65 ^{A,B,C}	0.000
WBCs (Thousand/mm ³)	24.21±1.15 ^{B,D}	30.89±1.81 ^{A,C,D}	28.63±1.21 ^{A,B}	27.32±1.88 ^B	0.000
Platelets (10 ⁴ /mm ³)	35.42±1.89 ^{B,C}	41.85±1.43 ^D	39.21±2.10 ^A	37.87±1.12 ^B	0.000

A, B, C, and D indicate significant differences in groups (ANOVA followed by post-hoc Tukey, $p \leq 0.05$)

Table-2: Serum Inflammatory markers levels in all groups (Mean±SD)

Test Parameters	Groups				p
	A	B	C	D	
Serum CRP (mg/dl)	0.11±0.09 ^B	0.85±0.19 ^{A,D}	0.61±0.21	0.29±0.38 ^B	0.002
Serum Fibrinogen (mg/dl)	231.5±68 ^{B,C}	450.3±0.37 ^{A,C,D}	292.3±0.19 ^{A,B}	321.2±0.51 ^B	0.000
IL-6 (pg/ml)	108.33±5.1 ^{B,C,D}	172.16±3.0 ^{A,C,D}	168.41±2.5 ^{A,B,D}	151.45±5.4 ^{A,B,C}	0.000
TNF-α (pg/ml)	105.2±5.1 ^{B,C,D}	276.4±6.3 ^{A,B,D}	221.8±5.5 ^{A,B,D}	158.6±4.8 ^{A,B,C}	0.000

A, B, C, and D indicate significant differences in groups (ANOVA followed by post-hoc Tukey, $p \leq 0.05$)

Table-3: Oxidative stress markers level across all groups (n=40)

Test Parameters	Groups				p
	A	B	C	D	
MDA (nmol/g tissue)	127.55±0.45 ^D	159.13±1.01	143.45±0.87 ^D	130.21±1.22 ^{A,C}	0.000
SOD (U/mL)	1129.36±100.23 ^{C,D}	213.71±0.21 ^{C,D}	714.19±49.27 ^{A,D}	937.31±28.74 ^{A,C}	0.000
GSH (mg/L tissue)	4.87±0.84 ^B	2.43±1.02 ^{A,D}	4.13±1.34	4.48±1.11	0.006

A, B, C, and D indicate significant differences in groups (ANOVA followed by post-hoc Tukey, $p \leq 0.05$)

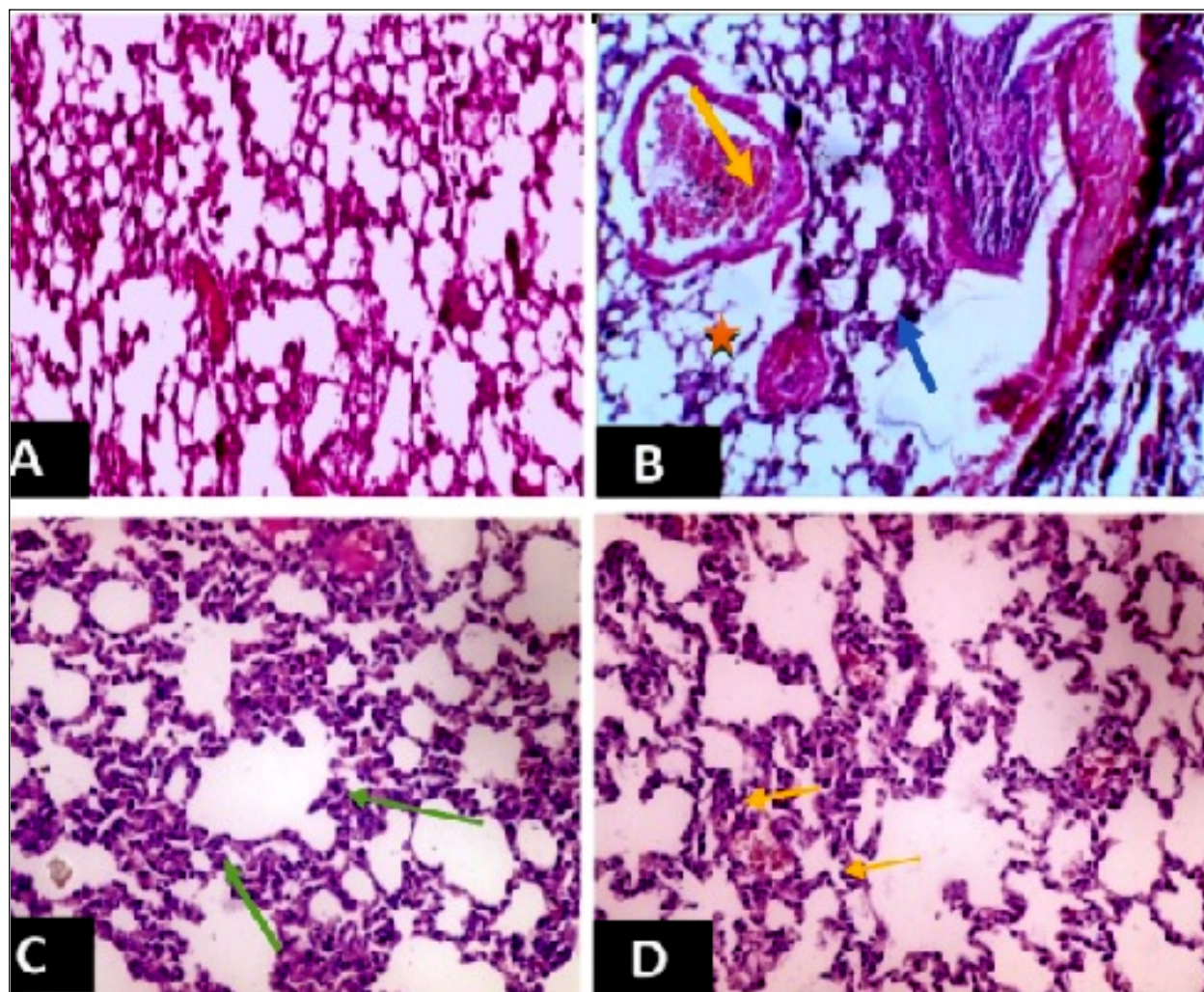


Figure-1: Histopathological microphotograph of lung tissue sections of all groups

DISCUSSION

Tobacco smoking is widespread worldwide. Nicotine, an ingredient in cigarettes, is harmful to human health and often causes an increase in mortality. Pineal gland's hormone melatonin is essential for preserving the health of both people and animals.

Our study demonstrated that there was a significant rise in RBCs, WBCs, Haemoglobin, and Platelets in the nicotine-induced Group B compared to the control group. This is due to the toxic effects of cigarette smoke on blood flow and vessel health as well as on blood elements such as WBCs and platelets.¹⁵ Melatonin-injected Groups C and D showed significant protection from the abnormally alterations of haematological parameters. This normalization is prominent in Group D compared to Group C. These findings are consistent with Wang *et al*¹⁶, Ngai *et al*¹⁷, Kulsoom *et al*¹⁸, and Zhao *et al*¹⁹, all of whom studied the potential protective effects of melatonin on lung tissues.

Our study observed elevated levels of CRP, Fibrinogen, IL-6 and TNF- α in the rats induced with nicotine only compared to all other groups. These findings are consistent with those reported by Siddiqui *et al*²⁰, Centner *et al*²¹, and Khaled *et al*²². According to the American Heart Association (AHA), cigarette smoking is linked with the expression of pro-inflammatory cytokines, particularly the activation of toll-like receptor 4 (TLR4)-inflammasome-IL-6 signalling axis, which in turn leads to an inflammatory cascade and ultimate release of different inflammatory cells and proteins including CRP and fibrinogen, etc.²³ The co-administration of melatonin in different doses decreased the levels of these inflammatory markers. This reduction was more pronounced in group D, though these levels did not return to control levels.

The ameliorative effects of melatonin against this raised level of inflammatory markers are reported by different studies.²³⁻²⁵ In addition to the anti-inflammatory properties, melatonin exhibits antioxidant potential against various toxic chemicals, especially nicotine. In the present study, we demonstrated that nicotine caused a significant decline in anti-oxidative markers in lung tissue. Countering this effect, treatment with 5 mg/Kg melatonin somewhat alleviated the nicotine-induced drop in MDA, SOD, and GSH activity. However, the decline was offset by co-administration of 10 mg/Kg of melatonin. Andersson *et al*²⁴, Lim *et al*²⁵, and Wang *et al*²⁶, reported the potential role of melatonin against the oxidative stress induced by nicotine in lung tissue.

The present study emphasizes how melatonin may have protective benefits in reducing the pulmonary tissue injuries caused by nicotine. Notwithstanding, it is imperative to recognize certain

constraints, such as the absence of evaluation of lipid profile, Cotinine levels, bronchoalveolar lavage and gene expression, which may have yielded more insights regarding the plausible anti-inflammatory and antioxidant properties of melatonin.

CONCLUSION

Higher doses (10 mg/Kg) of Melatonin have an effective property against inflammatory, oxidative and proliferative reactions resulting from nicotine in lung tissues. In cases of persistent nicotine-induced lung tissue damage, melatonin might be helpful. Further studies are recommended, including more parameters like lipid profile, and even gene studies, which will provide more in-depth findings.

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Received: 29 Jul 2025

Reviewed: 19 Feb 2026

Accepted: 25 Feb 2026

Contribution of Authors:

Authors approved the draft and are accountable in ensuring that questions related to accuracy or integrity of the work are duly investigated and resolved.

SPC: Concept, design, drafting, final approval

SK: Concept, design, data collection and assembly, drafting of article

SA: Concept, design, data collection and assembly, drafting of article

TFM: Analysis and interpretation of the data and drafting of article

QAS: Data collection and assembly, drafting of article

HA: Data collection and assembly, drafting of article

Conflict of Interest: No conflict of interest is declared

Funding: No funds have been received from any agency



ORIGINAL ARTICLE

EFFECT OF *BACILLUS CLAUSII* IN COMBINATION WITH PHOTOTHERAPY IN THE TREATMENT OF NEONATAL HYPERBILIRUBINEMIA: A QUASI-EXPERIMENTAL STUDY IN A TERTIARY CARE HOSPITAL

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Background: Neonatal hyperbilirubinemia is common in the first week of life, and probiotics like *Bacillus clausii* are gaining popularity in bilirubin clearance by modulating gut flora, and shown in previous studies to be safe and well-tolerated in newborns. The aim of this study was to compare the distribution of serum bilirubin levels between neonates receiving phototherapy alone and those receiving phototherapy plus *Bacillus clausii*. **Methods:** This quasi-experimental study using alternate allocation was conducted over six months from 1st Jan to 30th Jun 2025 in the Paediatric Department of PAF Hospital Mushaf, Sargodha. Eighty neonates were assigned to receive either phototherapy with *Bacillus clausii* (Group A) or phototherapy alone (Group B). Serum bilirubin levels were measured twice daily and finally evaluated on day four of treatment and compared between the groups. **Results:** Baseline characteristics were comparable between both treatment groups. Post-therapy serum bilirubin levels were significantly lower in the combined therapy group 'A' compared to the monotherapy group 'B' [median 4.0 (IQR 3.25–6.0) versus 6.0 (IQR 4.0–9.0); $p < 0.001$]. The effect size indicated a moderate treatment effect ($r = 0.40$), favouring the combined therapy approach. **Conclusion:** Combining *Bacillus clausii* with phototherapy appears to be a safe and effective approach for managing neonatal hyperbilirubinemia. This combination accelerates bilirubin clearance and may also help reduce the duration of phototherapy.

Keywords: *Bacillus clausii*, Bilirubin, Hyperbilirubinemia, Neonatal Jaundice, Phototherapy, Probiotics

Pak J Physiol 2026;22(1):33–6, DOI: <https://doi.org/10.69656/pjp.v22i1.1909>

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INTRODUCTION

Neonatal hyperbilirubinemia refers to the clinical state of elevated total serum bilirubin (TSB), which results in deposition of bilirubin into an infant's skin.^{1,2} It is one of the leading causes of admission to newborns' nurseries worldwide.³ It is characterized as yellowish discoloration of the skin, sclera, and mucous membranes.⁴ Approximately 60% of term and 80% of preterm neonates develop jaundice during the first 7 days of life,⁵ while nearly 10% of exclusively breastfed infants may develop breast milk jaundice.⁴ Neonatal hyperbilirubinemia can be physiological when it occurs due to increased red blood cell turnover, immature hepatic conjugation mechanisms, and enhanced enterohepatic circulation, or pathological which may arise from haemolysis, infection, metabolic disorders, or birth trauma.⁶ A number of cases are self-limiting, however in some cases, serum bilirubin may cross the blood-brain barrier, deposit in the basal ganglia, and cause permanent neurological damage, resulting in kernicterus.^{7,8}

Phototherapy remains the cornerstone of treatment for neonatal hyperbilirubinemia and effectively lowers serum bilirubin levels, thereby reducing the need for exchange transfusion.⁹ However,

phototherapy is associated with several limitations, including dehydration, temperature instability, mother-infant separation, and increased healthcare costs.¹⁰ Probiotics, particularly *Bacillus clausii*, have emerged as a potential therapeutic option due to their ability to modulate intestinal flora and reduce enterohepatic circulation of bilirubin via β -glucuronidase suppression.¹¹ Limited regional evidence exists regarding their effectiveness in the management of neonatal jaundice, warranting further investigation.

The rationale of this study was to fill the existing knowledge gap by investigating whether the combined use of *Bacillus clausii* and phototherapy offers clinically meaningful benefits in the management of neonatal jaundice. Specifically, the study aimed to determine whether this adjunctive therapy enhances bilirubin reduction compared with phototherapy alone, thereby potentially reducing the risk of bilirubin-induced neurological dysfunction, including kernicterus, while limiting the duration of exposure to phototherapy and its associated complications.

METHODOLOGY

This quasi experimental study was conducted in Paediatric Department, Pakistan Air Force Hospital Mushaf, Sargodha, after the approval of Ethical

Committee, from 1st Jan to 30th Jun 2025. Sample size was calculated using WHO Sample Size Calculator.¹² With population standard deviation= 38.5, confidence level=95%, and power of test=99%, total sample size calculated was 80 having 40 in each group.

Inclusion criteria was neonatal age ≤ 3 days, term neonates (gestational age ≥ 37 weeks), birth weight ≥ 2.5 Kg and the total serum bilirubin ≥ 15 mg/dL [hyperbilirubinemia defined as >250 $\mu\text{mol/L}$ (≈ 14.6 mg/dL) per NICE guidelines].¹³ The exclusion criteria included direct hyperbilirubinemia, infants of diabetic mothers, neonatal sepsis. Rh-incompatibility between mothers and neonates was also excluded; therefore, only Rh-compatible pairs (predominantly Rh⁺) were included in the study.

Informed consent was obtained from the primary caregivers of all babies before recruiting for study. Non-probability consecutive sampling technique was used, regardless of gender or race. Group allocation was done to ensure equal numbers of participants in each group; odd-numbered neonates were assigned to the combined treatment group and even-numbered neonates to the monotherapy group. Declaration of Helsinki was followed throughout the study.

Birth history and clinical examination was carried out when patient was screened for enrolment. Group A received *Bacillus clausii* 2,000 million spores per 5 ml once daily plus phototherapy up to 15 hours per day. Group B received only phototherapy. Neonates in both groups were on-demand feeding. Serum bilirubin level was assessed twice daily. Sampling was done when phototherapy lights were off to prevent photo-oxidation of the sample. Safety measures were implemented using a standardized nursing flow sheet to record hydration status, temperature, skin changes, and stool pattern, abdominal distension, vomiting, and feeding patterns in order to detect and manage any possible side-effect at the earliest. Serum total bilirubin levels were assessed at 4th day of treatment to detect the treatment outcomes.

Data were entered and analysed on SPSS-25. Descriptive analysis was done for all variables. The tests of statistical significance were Mann-Whitney U test for continuous variables (Shapiro-Wilk <0.05) and Pearson Chi-square test for categorical variables. Effect size was calculated to compare post-therapy outcomes between both the treatment groups. The $p \leq 0.05$ was considered statistically significant.

RESULTS

Both groups, Combined Therapy (A) and Monotherapy (B), included an equal number of participants (40 each). Continuous variables showed a non-normal distribution (Shapiro-Wilk $p < 0.05$). Baseline characteristics of participants were comparable (Table-1 and 2) between both the groups. Table-1 demonstrates the descriptive

analysis of continuous variables. Table-2 demonstrates the descriptive analysis of categorical variable.

No remarkable side-effects were observed in either treatment group. A statistically significant and clinically meaningful difference was observed in post-therapy serum bilirubin between the groups, showing the superiority of the combined therapy group (Table-3).

Table-1: Analysis of continuous variables

Study Variable	Group A	Group B	Mann-Whitney U (Z)	p (2-Tailed)
	Median (Inter-Quartile Range)*	Median (Inter-Quartile Range)*		
Age	3.0 (2.0–3.0)	3.0 (2.0–3.0)	780.00 (-0.240)	0.811
Gestational Age	39.5 (38.25–40.0)	39.0 (38.0–40.0)	613.00 (-1.840)	0.066
Baseline Serum Total Bilirubin	17.0 (17.0–19.0)	18.0 (16.25–19.0)	1593.500 (-0.261)	0.794

*Q1–Q3

Table-2: Analysis of categorical variables

	Group A (n=40)	Group B (n=40)	Chi-square (df)	p
Gender				
Males	23 (57.5%)	28 (70%)	1.352 (1)	0.245
Females	17 (42.5%)	12 (30%)		
Mode of Delivery				
SVD	17 (42.5%)	16 (40%)	0.52 (1)	0.82
LSCS	23 (57.5%)	24 (60%)		
Baby's Blood Group				
A ⁺	5 (12.5%)	7 (17.5%)	3.821 (3)	0.281
B ⁺	14 (35%)	10 (25%)		
AB ⁺	16 (40%)	12 (30%)		
O ⁺	5 (12.5%)	11 (27.5%)		
Mother's Blood Group				
A ⁺	6(15%)	7 (17.5%)	2.239 (3)	0.524
B ⁺	13 (32.5%)	14(35%)		
AB ⁺	15 (37.5%)	17 (42.5%)		
O ⁺	6 (15%)	2 (5%)		
Type of feeding				
Breast-feeding	15 (37.5%)	14 (35%)	0.054 (1)	0.816
Formula feeding	25 (62.5%)	26 (65%)		
History of jaundice among siblings				
Jaundice	11 (27.5%)	13 (32.5%)	0.238 (1)	0.62

Table-3: Post-therapy analysis of serum bilirubin level

Study Variable	Group A	Group B	Mann-Whitney U (Z)	p (2-Tailed)	Effect Size (r)
Median Post-therapy Serum Total Bilirubin	4.0 (3.25–6.0)*	6.0 (4.0–9.0)*	433.000 (-3.578)	<0.001	0.40

*Median (Interquartile Range: Q1–Q3)

DISCUSSION

The present study demonstrated the efficacy of combining *Bacillus clausii* with phototherapy in treating neonatal jaundice. Probiotics like *Bacillus clausii* are thought to enhance phototherapy outcomes by suppressing intestinal β -glucuronidase, thereby promoting bilirubin clearance,¹⁴ thereby promoting bilirubin clearance, preventing its reabsorption, and modulating the gut microbiota to accelerate bilirubin excretion.¹⁵

The baseline characteristics of both the treatment groups were comparable, indicating appropriate group allocation and minimizing potential confounding bias.

Waheed *et al.*, verified the efficacy of combining oral probiotics with phototherapy, they also noted comparable baseline characteristics between both groups, with a higher rate of vaginal deliveries.¹⁶ The baseline equivalence strengthens the internal validity of the study and supports that the observed treatment outcomes are likely due to the therapeutic interventions rather than underlying demographic or clinical differences.

In our study, combined therapy group showed greater improvement in serum bilirubin, decreasing from 17.0 mg/dL to 4.0 mg/dL, compared with 18.0 mg/dL to 6.0 mg/dL in the phototherapy-alone group. Our results are consistent with Eghbalian *et al.*¹⁷, who reported a mean (baseline) serum bilirubin of 15.6±1.7 mg/dL in the combination therapy group and 15.8±1.6 mg/dL in the monotherapy group, which decreased to 7.2±0.9 and 7.8±0.7 mg/dL respectively, supporting combination of phototherapy with oral probiotic therapy. Similar evidence is documented by Waheed *et al.*¹⁶, who recorded mean bilirubin at the time of admission as 18.14±2.35 mg/dL in the monotherapy group vs 19.86±5.85 mg/dL in the combined therapy group, which markedly improved after 72 hours, i.e., 12.31±2.29 mg/dL vs 10.75±2.23 mg/dL, supporting the synergistic benefit of phototherapy with oral probiotics.

Tariq *et al.*¹⁸ mentioned that duration of therapy was also decreased in combined therapy group vs monotherapy (3.13±0.92 vs 3.81±1.12 days; $p=0.002$). It highlights the importance of faster bilirubin reduction which in turn reduces risk of bilirubin encephalopathy, shortens hospital stay, and lowers phototherapy exposure.

Our study indicated a clinically meaningful effect size of 0.40. Quratul Ain *et al.*¹⁹ in their randomized controlled trial found combined treatment effectiveness as 53% vs only 35% in phototherapy alone group. Our findings are aligned with meta-analysis of Deshmukh *et al.*²⁰ who recorded a significant reduction in serum bilirubin level at 96th hour [MD: -1.74 (-2.92, -0.57); $p=0.004$] and 7th day [MD: -1.71 (-2.25, -1.17); $p<0.00001$; LOE: low] after probiotic treatment.

In contrast, Fatima *et al.*²¹ meta-analysis found a slightly better outcome with phototherapy alone (MD: 0.22; 95% CI: 0.19–0.26), possibly due to differences in probiotic strains, dosing, or population characteristics.

Overall, the findings of this study support that probiotics combined with phototherapy provide an effective approach to neonatal jaundice. Use of a specific probiotic strain (*Bacillus clausii*), clear IQR reporting, and high internal validity were the strengths of our study.

The limitations of our study included its single-centre design, lack of long-term follow-up for rebound jaundice, or the inability to control for exact breastfeeding frequency (which affects gut motility). While our results offer a clinical pathway to reduce hospital occupancy and neonatal stress, larger-scale multi-centre longitudinal studies are essential to establish standardized dosing protocols and evaluate long-term safety profiles.

CONCLUSION

Combining *Bacillus clausii* with phototherapy offers a safe, effective strategy for managing neonatal hyperbilirubinemia while reducing the risk of phototherapy-related complications.

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Received: 19 Oct 2025

Reviewed: 20 Mar 2026

Accepted: 31 Mar 2026

Contribution of Authors:

Authors approved the draft and are accountable in ensuring that questions related to accuracy or integrity of the work are duly investigated and resolved.

HBS: Drafting the work

AA: Analysis

AB: Interpretation of data

SM: Critical review

NB: Statistical analysis

SN: Data collection

Conflict of Interest: No conflict of interest is declared

Funding: No funds have been received from any agency



ORIGINAL ARTICLE

EFFECT OF KETOGENIC DIET ON LEPTIN LEVEL IN A DEPRESSED RAT MODEL

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Background: Depression is multidimensional disorder involving neuroendocrine disturbances. Several hormones are affected in depression, including gut related hormones such as leptin. Decreased leptin levels have been associated with worsening depressive symptoms and altered satiety. This study aimed to evaluate effect of ketogenic diet on leptin levels in rat model and its impact on depressive behaviour. **Methods:** This study included male Sprague Dawley rats (n=39) randomly divided into three equal groups (n=13/group) and housed at Khyber Medical University. Depression was induced using chronic restraint stress model by restraining rats for two hours daily for 21 days. Depression was confirmed using Forced Swim Test and Sucrose Preference Test. Group A served as depressed control group and was sacrificed after confirmation of depression. Group B received normal diet for four weeks, while Group C was administered ketogenic diet for four weeks. Ketosis was confirmed before sacrifice. Blood samples were collected through intra-cardiac puncture, centrifuged, and stored at -80 °C. Serum leptin levels were measured using ELISA. Behavioural tests were repeated to assess effect of dietary intervention. **Results:** Mean leptin levels were 0.92 ± 0.47 in depressed control group and increased to 1.32 ± 0.33 in ketogenic diet-treated depressed group ($p=0.019$). Forced Swim Test mobility significantly increased ($p=0.001$), while Sucrose Preference Test scores significantly improved ($p=0.026$) in ketogenic diet-treated depressed group. **Conclusion:** A four week ketogenic diet increased leptin levels and improved depressive behaviour in depressed rats.

Keywords: Depression, Forced swim test, Intra-cardiac puncture, Ketogenic diet, Ketosis, Leptin, Preference test, Sucrose re-strainer

Pak J Physiol 2026;22(1):37–41, DOI: <https://doi.org/10.69656/pjp.v22i1.1924>

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INTRODUCTION

Depression is a sophisticated disorder that disturbs both physical and mental aspects of individual's health. As individual suffering from depression manifest an extensive variations in their clinical symptoms, the exact underlying mechanism of its development remain incomprehensible.¹ However, many risk factors associated with cause of depression are identified in literature including female gender², poor socioeconomic level³, depression in past, dysthymia, any physical or cognitive disability and vascular factors, also diseases related to ageing⁴ can cause depressive symptoms, vascular inflammatory diseases, hormonal, and immune changes depress the integrity of fronto-striatal pathways and increase susceptibility to depression and the most important genes.⁵

Along with these, studies done recently has also shown strong association of microglial dysfunction with depression.⁶ Due to multiple risk factors prevalence of this disorder is increasing day by day and 34% of adolescents globally, aged 10–19 years, are at risk of developing clinical depression, which is exceeding the

reported estimates of individuals aged 18–25 years. Recent statistics of depression.⁷ Hormonal changes and depression are inter related, sometimes change in hormonal balance can lead to depression while otherwise depression can change hormonal balance in body, likewise change in appetite cycle is marked as a sign of depression due to imbalance of appetite hormones it was suggested in a study that higher depression score will lead to lower appetite score and *vice versa*.⁸ Now it is also a well-established fact that leptin plays a major role in satiety and effect of depression on satiety can be via changes in leptin levels in body. In a recent study it was clearly demonstrated that depression leads to lower level of leptin which in-turn stimulate intense hunger and appetite and leads to increased food consumption and then weight gain.⁹

Now to treat depression and altered leptin level many options are available, one of them is Ketogenic diet which is a low carbohydrate and high fat diet (70% fats, 27% proteins and only 3% carbs). Many studies in literature have shown that leptin levels gets better with Ketogenic diet and as a result satiety gets better and also depression.¹⁰

METHODOLOGY

This experimental study was carried on male Sprague-Dawley rats in KMU animal house after taking permission from ethical committee of the university under letter no KMU/IBM/IREB/16th. Our study included 39 (sample size was calculated according to resource equation method) healthy rats without any overt pathology weighing 200 to 250 grams. Rats were kept in cages having bedding of raw wood chips and were fed with water and normal food. Also rats facility was maintained on a 12:12 hour light n dark cycle under controlled temperature. Acclimatization period was kept seven days for them.

Afterwards to induce depression in rats they were kept in restrainers for 2 hours/day for 21 days according to chronic restrain stress model and depression was assessed and confirmed through FST (It is also known as behavioural despair test, is used to test for depression-like behaviour in rats) and SPT (It's a reward based test, used as an indicator of depression. This task assesses the animal's interest in a sweet-tasting sucrose solution relative to unsweetened water), all rats came out to be depression positive. At this point rats were randomly divided in three groups and first group (group A) was sacrificed through intra-cardiac puncture in post absorptive stage for baseline values of leptin. Collected blood was centrifuged immediately in laboratory and serum was stored at -80 °C in Eppendorf tubes. In the remaining 2 groups, Group B was fed with normal diet for 4 weeks while group C was on Ketogenic diet, then once ketosis was confirmed in group C rats through ketometer (Keto-Mojo GK+Blood Glucose and Ketone Monitoring System, Keto-Mojo, USA) both groups were sacrificed blood was collected and centrifuged at 3,000 rpm for 20 minutes using a laboratory centrifuge (Eppendorf 5702R, Hamburg, Germany) and at the same time the collected plasma was stored in freezer at -80 °C in Eppendorf tubes. Serum leptin levels were measured using a commercially available Rat Leptin ELISA Kit (Elabscience Biotechnology Inc., Wuhan, China; Catalog No: E-EL-R0582) following the manufacturer's protocol. Absorbance was measured using a microplate reader (BioTek ELx800, BioTek Instruments Inc., USA) at 450 nm, and concentrations were calculated from a standard calibration curve.

Data were analysed using SPSS-26. Quantitative variables were expressed as Mean±SD. Independent sample *t*-test was applied to compare differences between groups, and *p*<0.05 was considered statistically significant.

RESULTS

During the development of depression, the weight of the rats decreased in all the groups. Keto diet rat's weight

was decreased more as compared to the control diet group. There was a significant increase in leptin level (*p*=0.019) in depressed rats after being treated with Ketogenic diet (Table-1). Mobility of rats in FST was more in keto group (103.31±10.45 seconds) *p*=0.001 as compared to control group (69.62±29.51 seconds). Moreover, sucrose water intake was more in keto group (48.46±10.64 mg/mL) as compared to control rats (40.92±2.25 mg/mL) and test came out to be significant (*p*=0.026). For comparison of the groups, independent sample *t*-test was applied.

Table-1: Comparison of control and Keto group before and after intervention

Test Parameters	Control	Keto	<i>p</i>
Leptin (ng/L)	0.92±0.47	1.32±0.33	0.019
FST-mobility (Seconds)	69.62±29.51	103.31±10.45	0.001
FST-immobility (Seconds)	50.39±9.51	16.69±10.45	0.001
SPT(g/mL)	40.92±2.25	48.46±10.64	0.026
Ketones (mmol/L)	0.92±0.28	1.47±0.56	0.006
BSR (mg/dL)	177.92±146.34	147.23±86.83	0.522

DISCUSSION

Our study was to check if the Ketogenic diet intervention for four weeks has beneficial effect on depression scores and metabolic hormones. We found that four weeks of dietary intervention significantly decreased depression scores as measured in our study by FST and SPT. This was accompanied by more decrease in weight in the keto group. Leptin hormone was increased after Ketogenic intervention.

Leptin levels were significantly increased in depression. In contrast to our study it was reported that in depressive patients leptin levels are low as compared to controls and that is linked with primary reports of leptin's antidepressants role in animal models of depression.¹¹⁻¹⁴ Also on other hand there were results in literature showing parallel results with ours i.e., In any case, there was a strong linear association found between leptin levels and severity of depression. Higher leptin levels were found in patients with moderate to severe depression while lower levels in patients with mild depression.¹⁵ These differences in results may be based on difference in gender, age group, difference in diets in populations under study.

The pathophysiology of depression is complex and involves intricate interaction between biological, psychological and environmental factors. Although exact mechanism of depression is still not known some of the contributing factors could be neurotransmitter dysfunction particularly serotonin, nor epinephrine and dopamine, other could be neuroendocrine dysregulation specially when HPA axis is dysregulated, thirdly could be neuroinflammation like activation of pro inflammatory cytokines can lead to depression.¹⁶ Psychological and social stressors like childhood trauma, isolation and stress can be a leading cause of

depression. Lastly genetic and epigenetics could be one of major causes and now they are the domains of most active research nowadays in relation to depression.¹⁷

The effects of Ketogenic diet on leptin in depress rat model have sparked considerable interest within the realm of neuroscience and metabolic research. Leptin often referred as satiety hormone plays a crucial role in regulating appetite, metabolism and energy expenditure has also emerged as a potential mediator of mood disorders including depression.

Leptin is primarily produced by adipose tissues and acts on the hypothalamus in the brain to suppress appetite and increase energy expenditure. When leptin levels are sufficient, it signals to the brain that the body has adequate energy stores, thus reducing hunger and promoting weight maintenance. However leptin resistance can occur, wherein the brain becomes less responsive to leptin's signals, leading to increased appetite and weight gain.¹⁸

Studies on rodents subjected to Ketogenic diet have shown mixed results regarding leptin levels and depressive symptoms.¹⁹ Some research indicates that Ketogenic diet may lead to reduced levels of leptin, suggesting a potential mechanism for appetite suppression and weight loss. However the implications of reduction in this leptin on depressive symptoms remain unclear.²⁰ Similarly in other study low level of leptin have been associated with depressive symptoms, mentioning that individuals with depression tend to have lower circulating levels of leptin.²¹

On the other hand, there is evidence suggesting that keto diet could have antidepressants effects in animal models mediated through alterations in neurotransmitter level, mitochondrial function, rather than solely through changes in leptin level.²² The discrepancy in findings may be attributed to difference in experimental protocol, difference in gender, age group, difference in diets composition, duration of exposure and animal models used.

The Ketogenic diet, characterized by its high fat, moderate protein and low carbohydrate has gained popularity for its potential to promote weight loss and improve metabolic health. One intriguing aspect of Ketogenic diet is its influence on hormone levels including leptin. Research on impact of Ketogenic diet on leptin level is still evolving but several studies has provided valuable insights into this relationship. Initially some studies suggested that Ketogenic diet may lead to a decrease in leptin levels potentially due to decrease carbs intake and subsequent change in insulin levels.²³ Since insulin can stimulate leptin secretion, lower insulin level in keto diet might result in decreased leptin production. Additionally changes in adipose tissue mass and distribution, particularly reduction in visceral fats, may impact leptin secretion.

However, recent studies have reported a counter-intuitive rise in leptin levels in individuals adhering to Ketogenic diet. Several factors may contribute to the increased levels of leptin firstly could be unique metabolic effects, characterized by ketosis and increased fat oxidation could play a major role. Ketone bodies, produced during ketosis, have been shown to influence leptin secretion and sensitivity, potentially leading to elevated leptin level. Moreover changes in dietary composition, particularly the high fat content of the Ketogenic diet, could impact adipose tissue metabolism and leptin production.²⁴

The animal model used in this research was based on chronic restraint model which allows investigating physiological, behavioural and neurobiological changes induced by chronic stress exposure. This model involves subjecting animals, typically rodents such as rats or mice, to repeated periods of physical immobilization or confinement. Animals are placed in restraining device for several hours daily over a period ranging from days to weeks. The stressors are applied consistently, mimicking persistent stress experienced in real life situations.

Physiologically, chronic restraint stress elicits a cascade of responses in animals. These include alteration in HPA axis or even dysregulation of cortisol in case of prolonged stress. Similarly behaviourally, chronic restraint stress induces a range of adoptive and maladaptive responses. While some animals may exhibit anxiety like behaviour, like increased vigilance, others may display depressive like behaviour like reduced motivation and anhedonia. Cognitive impairments, like deficits in learning and memory, have also been observed in animals exposed to chronic stress.

The chronic restraint stress model has provided valuable insights into the pathophysiology of stress related disorders, including depression, anxiety disorder, and post-traumatic stress disorder (PTSD). By elucidating the neurobiological mechanisms underlying stress induced changes, researchers can identify potential targets for pharmacological interventions and behavioural therapies aimed at mitigating the adverse effects of chronic stress.²⁵

The neurobiological mechanisms underlying the effects of chronic restraint stress are multifaceted and involve complex interactions between brain regions and neurotransmitter systems. Key brain areas implicated in stress response include the prefrontal cortex, amygdala and hippocampus, which regulate emotional processing, fear responses, and cognitive function, respectively.²⁶ Stress can not only induce structural and function changes in these brain areas but also cause dysregulation of neurotransmitter system such as serotonin, dopamine etc and may cause mood disorders including anxiety and depression.

Animal model play crucial role in depression research, offering insights into the biological and behavioural underpinning of disorder. One such model is the force swim test, traditionally used to assess antidepressant efficacy in rodents. However recent studies have shown its potential as diagnostic tool for depression in animal models.²⁷ This test involves placing a rodent in a cylindrical container filled with water from which escape is impossible. Initially animal exhibits vigorous attempts to escape but eventually adopts a state of immobility, floating passively. This immobility is interpreted as measure of behavioural despair. Studies have shown that animal susceptible to depression display prolonged immobility times compared to resilient counterparts. Furthermore these depressive like behaviours often correlate with other hallmark symptoms of depression, such as anhedonia and social withdrawal.²⁸

While FST offers a valuable tool for depression research, several challenges must be addressed to ensure its validity and reliability as a diagnostic model. Variability in experimental conditions such as water temperature, container size, lighting, can influence test outcomes and complicate result interpretation.

Another model which play role in understanding the neurobiological mechanisms underlying depression is sucrose preference test. This model is based on the principle that rodents, like humans, exhibit a preference for sweet solution such as sucrose when given a choice between sucrose solution and water. Anhedonia, a core symptom of depression characterized by diminished ability to experience pleasure, is reflected in reduced preference for sucrose consumption. The test typically involves two phases: habituation to sucrose solution followed by a period of preference testing. Rodents subjected to chronic stress often exhibit decreased sucrose preference compared to control counterparts. This reduction in sucrose preference is interpreted as anhedonia, indicative of depression like state in animals.²⁹

Our study revealed relation of Ketogenic diet with depression and leptin. However further research is needed to elucidate the precise mechanisms underlying the effects of Ketogenic diet on leptin level and depressive symptoms in animal model, as understanding the interplay between keto diet, depression and leptin in animal models could provide valuable insights into potential therapeutic strategies for mood disorder in humans. We can apply this model on humans and check the results. Duration of study can be increased in order to stop keto diet in keto group and fed them again with normal food and then check levels of leptin that what keto has done in long term. This project can be used on very young and aged rats to check effects of keto diet on depression and leptin level in extreme ages.

CONCLUSION

Ketogenic diet for four weeks increased leptin significantly in rats. Keto diet significantly improved depression scores as assessed by force swim test and sucrose preference test.

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Received: 22 Jan 2026

Reviewed: 21 Mar 2026

Accepted: 31 Mar 2026

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SMSB: Data entry and analysis, Tabulation, Writing and Revision of manuscript	SA: Data Analysis, Tabulation, Writing and Revision of manuscript
MIS: Data analysis, Writing and Revision of manuscript	AH: Data analysis, Writing and Revision of manuscript
AI: Tabulation of Results, Writing and Revision of manuscript	SI: Data analysis and Tabulation, Writing and Revision of manuscript
MM: Data analysis and Tabulation, Writing and Revision of manuscript	MM: Data analysis and Tabulation, Drafting and Revision

Conflict of Interest: The authors declare that they have no conflicts of interest or competing interests

Funding: This work was partially supported by a research grant awarded through the Office of Research and Innovation, Khyber Medical University, Peshawar, Pakistan



ORIGINAL ARTICLE

WHOLE EXOME SEQUENCING OF A PAKISTANI FAMILY SEGREGATING AUTOSOMAL RECESSIVE GAUCHER DISEASE TYPE I

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Background: Gaucher Disease (GD) is a rare inherited lysosomal storage disorder caused by mutations in the *GBA1* gene on chromosome 1q21. This study aimed to perform clinical and genetic evaluation of two patients with clinically suspected Gaucher's disease and to identify the underlying pathogenic *GBA1* variants. **Methods:** Two patients from a consanguineous Pakistani family were admitted with developmental delay and hypotonia. Detailed physical examination was performed. Complete blood count (CBC), Liver Function Tests (LFTs), Inflammatory markers, Erythrocyte Sedimentation Rate (ESR), and radiological procedures including ultrasound and MRI were advised. Later, Whole Exome Sequencing (WES) followed by DNA Sanger sequencing was performed. **Results:** Analysis of complete blood count demonstrated features of microcytic, hypochromic anaemia in the patients. No abnormalities were observed in liver function tests or erythrocyte sedimentation rate. Physical examination revealed hypotonia. Radiological analysis showed hepatosplenomegaly. Genetic analysis identified a homozygous missense mutation, c.1448T>C; p.Leu483Pro, in the *GBA* gene. **Conclusion:** This study confirms Gaucher disease in two Pakistani patients with a homozygous *GBA1* variant and highlights the importance of integrating clinical assessment with genetic testing for accurate diagnosis.

Keywords: Bone Marrow, Gaucher Disease, *GBA1* Gene, Genetic Analysis, Haematological Tests

Pak J Physiol 2026;22(1):42–5, DOI: <https://doi.org/10.69656/pjp.v22i1.1925>
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INTRODUCTION

Philip Gaucher, a French dermatologist, in 1882, identified an abnormality in a patient with splenomegaly, later named Gaucher cells with condensed chromatin and eccentric nucleus. Later, the disease was termed 'Gaucher Disease'. The Gaucher cells are, in fact, abnormal macrophages, preferential targets of this rare autosomal and recessive genetic disease.^{1,2} Gaucher disease (GD) is the most common lysosomal storage disorder worldwide. It is an autosomal recessive condition caused by mutations in the acid β -glucosidase gene (*GBA1*; OMIM 606463) located on chromosome 1q21, resulting in deficiency of the lysosomal enzyme β -glucocerebrosidase.^{3,4} This deficiency leads to accumulation of glucocerebroside within lysosomes, particularly in the spleen, liver, lungs, and brain, forming characteristic Gaucher cells.^{5–7} GD is classified as a lysosomal sphingolipidosis and is reported as one of the more frequent genetic disorders in certain populations.^{5,6}

Based on clinical presentations, severity, and age of onset, GD can be categorized into three subgroups. Type 1 GD with anaemia, chronic and non-neuropathic, and massive hepatosplenomegaly is observed in early adulthood. Type 2 GD is characterized by progressive neurological weakening with hepatosplenomegaly. Type-3 GD is the juvenile form with onset between 2–6 years. The most common

presentation of this type is hepatosplenomegaly along with a moderate level of neurological symptoms. Skeletal deformities are observed with the progression of age.⁸

Gaucher Disease associated with pulmonary involvement is rare but has been reported in limited case series. The literature review revealed only one case of GD with itching.^{8,9} In the current study, we report a highly consanguineous Pakistani family segregating a rare type of Gaucher Disease with potential risk for neuropathic progression. Genetic analysis of the *GBA1* (OMIM, 230800) gene revealed an already known homozygous variant p.Leu483Pro, which is rare in the Pakistani population.

METHODOLOGY

A 1.5 years old male (P1) and a 3 years old (P2) female patient suffering from GD-type 1 were enrolled in this study. Both patients belonged to consanguineous parents from a remote area of Lahore. The indexed patients were brought to the Department of Paediatrics at the Pakistan Air Force Hospital, Islamabad.

The study was approved by the Ethical Review Committee of HBS Medical College, Islamabad vide No. HBS/IRB/19/25, Fazaia Medical College vide IBD/FMC/1320/01/PHY, M. Islam Medical College vide No. 1/2026/Bio/MIMDC, and Nishtar Medical College Multan vide No. 02/2026/PHY/NMU. Before taking the patients' history, informed written consent

was obtained from the father of patients.

The indexed male patient presented yellow spots in the eye and a swollen belly. The mother also complained of body aches and lethargy. During family history, the mother reported that the elder sibling, a 3-year-old female (P2), had similar symptoms. She also shared the laboratory reports previously conducted for P2. The index patient was then evaluated by an expert paediatric team to exclude possible infections, particularly those involving the gastrointestinal tract. A geneticist suspected a case of genetic disease and required all the important information related to family history, patient clinical presentations, and the onset of the disease. A pedigree was generated based on the patients' family history.

The physician initially advised complete blood count (CBC), liver function tests (LFTs), and abdominal ultrasound to evaluate splenomegaly. Based on clinical presentation of hepatosplenomegaly and constitutional symptoms, lymphocyte subset analysis and immunoglobulin levels were included in the diagnostic workup to exclude haematological malignancies and lymphoproliferative disorders. Previous laboratory findings of P2, including CBC and lymphocyte subset analysis, were also reviewed.

For laboratory analysis, 5 mL whole blood samples were collected in EDTA/heparin tubes (Becton, Dickinson and Company, USA) for CBC, flow cytometry, immunophenotyping, and DNA extraction. For LFTs, samples were collected in serum separator (gold-top) tubes. All tests were carried out at Armed Forces Institute of Pathology and Immunology Department, CMH, Rawalpindi. Flowcytometry and liver function tests were performed according to the protocols reported in our earlier publication.¹⁰

RESULTS

Lymphocyte subset analysis showed relative lymphocytosis with altered T- and B-cell distribution, while NK cell counts were reduced in both patients (P1 and P2) (Table-1). The complete blood count in the patient (P1) revealed leukopenia, anaemia, and low platelet count in P1 and P2 (Table-2). Moreover, differentials revealed high ESR and reticulocyte count (Table-3). Bone marrow aspirates showed hyperplastic erythropoiesis and depressed myelopoiesis in P1. Megakaryocytes were increased with marked dysplasia in P1 (Table-4). Liver function tests showed mildly elevated ALT and AST, while albumin and bilirubin were within the reference range in patient 1.

Pedigree analysis indicated an autosomal recessive pattern of inheritance (Figure-1A). The affected individuals include two siblings, IV-1 and IV-2. Their parents (III-1 and III-2) are clinically healthy but are likely carriers of the condition. One cousin (IV-3) is unaffected and shows no clinical features of the disease.

Whole-Exome-Sequencing (WES) was performed in the male patient (P1, IV-4). There were many single nucleotide polymorphisms (SNPs), identified in the patients. An interesting pathogenic homozygous missense variant c.1448T>C; p.Leu483Pro was identified in chromosome 1q21 in the gene Glucosylceramidase Beta (*GBA*) (Figure-1B). The segregation of the missense variant was confirmed through DNA Sanger sequencing. Mutations in the *GBA* gene are reported to cause Gaucher's Disease.

Table-1: Lymphocyte subset analysis [n (%)]

Test Name	P1	P2	Reference Range
TLC (/μL)	4,500	4,700	4,000–12,000
Lymphocytes (%)	72	70	44–74
CD3+ cells	1,652 (51)	1,554 (48)	2,100–6,200 (53–75)
CD3+ CD4+ cells	875 (27)	939 (29)	1,300–3,400 (32–51)
CD3+ CD8+ cells	518 (16)	485 (15)	620–2,000 (14–30)
CD19+ cells	1,458 (45)	1,198 (37)	720–2,600 (16–35)
CD16+ CD56+ cells	97 (3)	161 (5)	180–920 (3–15)
CD4:CD8	1.6	1.6	1.3–3.0

Table-2: Blood complete picture

Test	P1	P2	Reference range	
			Male	Female
Total leukocytes (10 ⁹ /L)	4.77	5.51	4.0–10.0	4.0–10.0
RBC count (10 ¹² /L)	3.94	3.18	4.5–6.3	3.8–5.2
Haemoglobin (g/dL)	9.3	8.8	14–18	11.7–15.2
Haematocrit (%)	0.28	0.31	0.39–0.49	0.35–0.48
MCV (fL)	71.8	70.3	77–91	77–91
MCH (pg)	23.5	24.5	26–32	26–32
MCHC (g/dL)	32.7	33.7	32–36	32–36
Platelet counts (10 ⁹ /L)	40	48	150–400	150–400
Absolute Neutrophil count (10 ⁹ /L)	0.95	0.97	1.5–7.7	

Table-3: Differential analysis of blood

Test	P1	Reference range
Neutrophils (%)	20	40–75%
Lymphocytes (%)	75	20–45%
Monocyte (%)	4	2–10%
Eosinophils (%)	1	1–6%
ESR (mm/1 st Hour)	59	(Male: 0–15, Female: 0–20)
Reticulocytes	4.5	(0.5–2%)
Red Cell Morphology		
Anisocytosis		-
Microcytosis		+
Poikilocytosis		+
Macrocytosis		-
Hypochromia		-
NRBCs		1/100 WBCs

Table-4: Bone marrow aspiration analysis

Site (s)	Posterior superior iliac spine
Consistency of bone	Normal
Cellularity	Normal Cellular Marrow
Erythropoiesis	Hyperplastic, Normoblastic
Myelopoiesis	Depressed
M:E Ratio	<1
Blasts	Nil
Megakaryocytes	Increased with marked dysplasia
Lymphocytes	Increased 60%
Plasma cells	Normal
Abnormal cells	A few scattered storage cells with crumpled tissue paper appearance seen

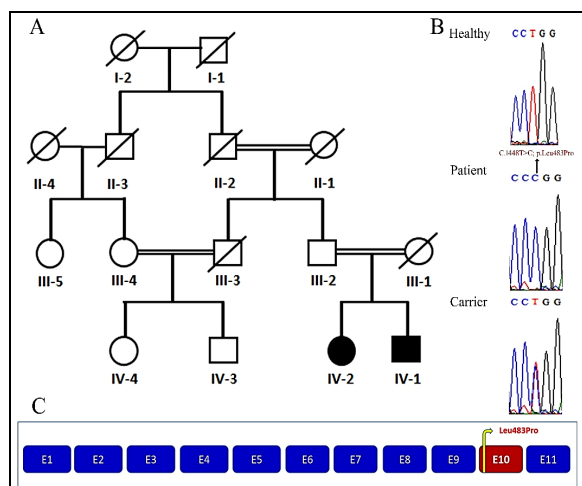


Figure-1: A: represent pedigree chart. Filled circles and squares represent female and male patients, while a double line indicates cousin marriage. B: represents Sanger sequencing of the target patient. Arrowhead showing site of mutation c.1448T>C; p.Leu483Pro. The mutation is segregating in healthy individuals and carriers. C: *GBA* gene structure showing 11 exons with site of mutation in exon 10

DISCUSSION

In humans, inherited Gaucher Disease type I (GD1, OMIM 230800) is a relatively common metabolic disease related to lysosomal storage caused by mutations in the *GBA* gene. *GBA* gene deficiency reveal variety of disease phenotypes which are grouped into type 1 (Non-neuronopathic), type 2 (Acute neuronopathic), and type 3 (Chronic neuronopathic). Data on Gaucher disease prevalence in Pakistan are limited, with higher occurrence observed in consanguineous families. In general population the incidence rate is ~1.5 per 100,000 live births, while specifically in the Pakistani population (Selected population) the frequency of GD is 34.4%.^{11,12} The gene *GBA* encodes a 497 amino acid protein, glucocerebrosidase (GCase), a key enzyme required for the breakdown of glucocerebroside, a cell membrane lipid.¹³ To date, more than 300 point mutations, large deletions, and insertions have been reported in the gene *GBA*.¹⁴

The severity of the disease manifestations depends on the type of mutation. Hence, the genetic makeup and the severity of the disease in GD patients are complex genotype–phenotype correlations. In a few cases, patients with similar mutations may exhibit different symptoms of GD. Genetic, Epigenetic, and environmental factors may influence disease expression.^{15,16}

In the current study, we performed genetic analysis in two patients suffering from GD belonging to a highly consanguineous Pakistani family. Whole

Exome Sequencing revealed a missense mutation converting nucleotide Cytosine to Thymine at position 1448, resulting in a change of amino acid Leucine to Proline 483 [c.1448T>C; p.Leu483Pro]. This *GBA* gene mutation in the Iranian population was recently reported by Mozafari *et al*¹⁷ and Youssef *et al*¹⁸. In earlier reports, *GBA* gene variant p.Leu483Pro was reported in both homozygous as well as compound heterozygous states with type 2 and type 3 GD, respectively.¹⁸ In GD patients, the genotype-phenotype correlation is not absolute. The GD patients with the p.Leu483Pro mutation require close monitoring owing to their subtle course and potential late neurological disease phenotypes. In our patients, we observed hepatosplenomegaly and cytopenia with currently no neurological manifestations. In most cases, the neurological manifestations develop late in life. Long-term follow up reveal the development of supranuclear gaze palsy and cognitive impairment. The late onset of neurological manifestations is also observed in patients with enzyme replacement therapy.¹⁹

The gene *GBA* has approximately 7.6–8 kb of genomic sequence and comprises 11 exons. The encoded protein GCase comprises three major domains: domain-I, domain II, and domain III. The mutation identified in the current report is located in exon 10 of the *GBA* gene. This mutation lies in domain II of the protein and is predicted to affect proper protein folding, stability, and protein–protein interaction. Like other *GBA* mutations, p.Leu483Pro is also associated with neurological manifestations linked to GD with increased risk of Parkinson's disease.²⁰

CONCLUSION

This study reports two consanguineous Pakistani patients with Gaucher disease associated with the p.Leu483Pro mutation, which carries a potential risk for neuronopathic progression. Both patients presented with hepatosplenomegaly and cytopenia. These findings broaden the genetic and clinical spectrum of Gaucher disease in Pakistan and highlight the importance of molecular diagnosis and long-term follow-up to monitor for possible late-onset neurological involvement.

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Received: 15 Jan 2026

Reviewed: 22 Mar 2026

Accepted: 31 Mar 2026

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SKA: Critical review and Clinical evaluation

SA: Clinical investigation

SIR: Critical review and Final approval for submission

Conflict of Interest: No conflict of interest is declared,

Funding: No funds have been received from any agency



ORIGINAL ARTICLE

RIGHT VENTRICULAR FUNCTION ON ROUTINE ECHOCARDIOGRAPHY AS A PREDICTOR OF SHORT-TERM OUTCOMES IN ACUTE HEART FAILURE ADMISSIONS IN A RESOURCE-LIMITED TERTIARY CARE HOSPITAL IN PAKISTAN

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Background: Acute heart failure (AHF) is associated with high short-term morbidity and mortality. While left ventricular function is routinely evaluated, right ventricular dysfunction has emerged as an important determinant of outcomes. Simple echocardiographic parameters such as tricuspid annular plane systolic excursion (TAPSE) and right ventricular fractional area change (FAC) may provide useful prognostic information in patients hospitalized with AHF. The objective of this study was to evaluate right ventricular function using TAPSE and FAC and evaluate their association with short-term clinical outcomes in patients with acute heart failure. **Methods:** This prospective observational study included 120 patients admitted with acute heart failure at a tertiary care Cardiology Unit. Transthoracic echocardiography within 24 hours assessed left ventricular ejection fraction (LVEF), TAPSE, FAC, and pulmonary artery systolic pressure. Right ventricular dysfunction was defined as TAPSE <16 mm or FAC <35%. Patients were monitored during hospitalization for short-term outcomes including ICU admission, inotropic support, mechanical ventilation, worsening renal function, prolonged hospital stay, and in-hospital mortality. **Results:** The mean age of patients was 54.62±8.15 years and 65.8% were male. Reduced TAPSE and FAC were observed in 46.7% patients. ICU admission, need for inotropic support, and mechanical ventilation were significantly more frequent in patients with reduced TAPSE and FAC ($p \leq 0.01$). Prolonged hospital stay was significantly associated with reduced FAC ($p < 0.05$). Echocardiographic differences between survivors and non-survivors were not statistically significant. **Conclusion:** RV dysfunction is associated with adverse short-term outcomes in AHF and may aid in early risk stratification.

Keywords: Acute heart failure, echocardiography, fractional area change, right ventricular function, TAPSE

Pak J Physiol 2029;22(1):46–51, DOI: <https://doi.org/10.69656/pjp.v22i1.1942>
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INTRODUCTION

Acute heart failure (AHF) represents a complex clinical syndrome characterized by the rapid onset or worsening of symptoms and signs of cardiac dysfunction, leading to urgent hospital admission and high short-term morbidity and mortality.¹ While left ventricular (LV) dysfunction has traditionally been the primary focus in heart failure assessment, growing evidence highlights the prognostic importance of right ventricular (RV) function.² The right ventricle plays a critical role in maintaining effective pulmonary circulation and overall cardiac output, particularly under conditions of increased haemodynamic stress.³ Simple echocardiographic indices such as tricuspid annular plane systolic excursion (TAPSE) and fractional area change (FAC), obtained during routine transthoracic echocardiography, offer practical means for assessing RV systolic performance.⁴

Heart failure remains a major global health burden, affecting more than 64 million individuals worldwide and accounting for substantial healthcare utilization. Acute decompensated episodes are among the

leading causes of hospitalization in adults over 65 years of age and are associated with high early readmission and mortality rates. In low- and middle-income countries, including South Asia, the burden of AHF is increasing due to population aging, rising prevalence of hypertension, ischemic heart disease, and limited access to advanced heart failure therapies.⁵ The aetiology of acute heart failure is heterogeneous and includes ischemic heart disease, hypertensive crises, cardiomyopathies, valvular heart disease, and arrhythmias.⁶ Common precipitants include myocardial ischemia, uncontrolled blood pressure, infection, non-adherence to medication, and renal dysfunction. Several risk factors contribute to adverse outcomes in AHF, including advanced age, diabetes mellitus, chronic kidney disease, pulmonary hypertension, and pre-existing right ventricular dysfunction. Importantly, conditions that increase pulmonary vascular resistance or RV afterload disproportionately affect right ventricular performance and may worsen short-term prognosis.⁷

From a pathophysiological perspective, RV dysfunction in AHF results from complex interactions between pressure overload, volume overload,

myocardial ischemia, and ventricular interdependence. Acute elevations in left-sided filling pressures can transmit backward into the pulmonary circulation, increasing RV afterload and impairing RV systolic function.⁸ The interventricular septum and pericardial constraint further link LV and RV performance, such that LV failure can precipitate or exacerbate RV dysfunction. Impaired RV contractility leads to reduced forward flow, systemic venous congestion, hepatic and renal dysfunction, and diminished response to standard therapies.⁹

Current management of AHF focuses on haemodynamic stabilization, relief of congestion, optimization of preload and afterload, and treatment of underlying precipitants. Although guideline-directed medical therapy primarily targets LV dysfunction, recognition of RV impairment has important therapeutic implications, particularly regarding fluid management, use of diuretics, vasodilators, and inotropic support.¹⁰

Existing literature increasingly supports the prognostic value of RV dysfunction in both chronic and acute heart failure; however, its routine integration into risk stratification remains inconsistent. Variability in measurement techniques, patient populations, and outcome definitions has led to ongoing debate regarding the optimal parameters and thresholds for RV assessment. In this context, easily accessible echocardiographic markers such as TAPSE and FAC continue to attract attention for their potential role in identifying high-risk patients during acute heart failure admissions and informing early clinical decision-making. The objective of this study was to evaluate right ventricular function using TAPSE and FAC and evaluate their association with short-term clinical outcomes in patients with acute heart failure.

MATERIAL AND METHODS

After obtaining a formal approval from the Institution Research and Ethical Board (IREB), this prospective observational study was conducted in the Department of Cardiology of a tertiary care teaching hospital, Peshawar. A total of 120 consecutive patients admitted with a diagnosis of acute heart failure (AHF) were enrolled during the study period from Apr to Sep 2025. Acute heart failure was defined as new-onset or worsening signs and symptoms of heart failure requiring hospital admission, based on clinical evaluation supported by laboratory and radiological findings.

Patients aged 18 years or older of either gender were included in the study. Patients with congenital heart disease, significant primary pulmonary disease, poor echocardiographic window, prior cardiac surgery affecting right ventricular geometry, or haemodynamic instability precluding echocardiographic assessment were excluded.

After obtaining informed written consent, detailed demographic and clinical data were recorded at the time of admission. These included age, gender, and co-morbid conditions such as hypertension, diabetes mellitus, ischemic heart disease, and chronic kidney disease, along with vital signs and baseline laboratory investigations.

All patients underwent routine transthoracic echocardiography (TTE) within 24 hours of admission using a standardized protocol. Right ventricular systolic function was assessed using tricuspid annular plane systolic excursion (TAPSE) measured in the apical four-chamber view with M-mode, and right ventricular fractional area change (FAC) calculated by tracing the right ventricular endocardial borders in systole and diastole. Reduced right ventricular function was defined according to guideline-recommended cut-off values (TAPSE <16 mm and FAC <35%). Left ventricular ejection fraction (LVEF) and other relevant echocardiographic parameters were also recorded.

Patients were managed according to standard hospital protocols for acute heart failure, and treating physicians were blinded to the study-specific analysis. Short-term clinical outcomes were recorded during hospitalization, including in-hospital mortality, intensive care unit (ICU) admission, requirement of inotropic support, mechanical ventilation, worsening renal function, and length of hospital stay. Patients were followed until hospital discharge or death.

The sample size was calculated using calculator.net, sample size calculator for proportion estimation, assuming an expected adverse outcome frequency of 30%, a 95% confidence level, and an 8% margin of error. The minimum calculated sample size was 126 patients; however, 120 consecutive eligible patients were included in the final analysis.

Data were analysed using SPSS-29. Continuous variables were expressed as Mean $\pm$ SD, while categorical variables were presented as frequencies and percentages. Associations between categorical variables were analysed using the Chi-square test while independent sample *t*-test was used for comparison of continuous variables between groups, and $p \leq 0.05$ was considered statistically significant.

RESULTS

The study included a total of 120 patients with acute heart failure. The mean age of the participants was 54.62 $\pm$ 8.15 years. Majority of patients were younger than 60 years, accounting for 87 (72.5%) cases. Male patients were 79 (65.8%) and 41 (34.2%) were females. Hypertension was present in 98 (81.7%) patients. Diabetes mellitus was observed in 31 (25.8%) patients. Ischemic heart disease was documented in 44 (36.7%) participants. Chronic kidney disease was present in 14 (11.7%) patients. (Table-1).

Echocardiographic assessment at the time of admission demonstrated a mean left ventricular ejection fraction of $36.22 \pm 4.07\%$. The mean tricuspid annular plane systolic excursion was 17.71 ± 3.92 mm, with more than half of the patients (64, 53.3%) showing TAPSE values greater than 16 mm, while reduced TAPSE of <16 mm was observed in 56 (46.7%) patients. The mean right ventricular fractional area change was $34.90 \pm 6.76\%$, with 64 (53.3%) patients having FAC values above 35% and 56 (46.7%) demonstrating FAC below 35%. Pulmonary artery systolic pressure was elevated, with a mean value of 49.78 ± 7.64 mmHg across the study population. (Table-2).

In-hospital mortality was observed in 14 (11.7%) patients. Admission to the intensive care unit was required in 38 (31.7%) patients. Inotropic support was needed in 44 (36.7%) cases. Mechanical ventilation was instituted in 26 (21.7%) patients. Worsening renal function during hospitalization occurred in 38 (31.7%) patients. Prolonged hospital stay of more than seven days was noted in 60 (50.0%) patients, with an equal proportion discharged within 7 days. (Table-3).

Among patients who did not require ICU admission, 52 (63.4%) had TAPSE >16 mm, while 30 (36.6%) had TAPSE <16 mm. In contrast, ICU admission was more frequent among patients with reduced TAPSE, with 26 (68.4%) having TAPSE below 16 mm compared to 12 (31.6%) with TAPSE above 16 mm. Regarding inotropic support, the majority (65.8%) of patients who did not require inotropes had TAPSE >16 mm, whereas those (30, 68.2%) requiring inotropic support more commonly exhibited reduced TAPSE. Eighteen (69.2%) patients who required mechanical ventilation predominantly had TAPSE <16 mm, compared to those (8, 30.8%) with preserved TAPSE. A higher proportion of patients (22, 57.9%) developing renal deterioration had TAPSE <16 mm, although this association did not reach statistical significance. (Table-4).

Among patients who did not require ICU admission, 52 (63.4%) had FAC values $>35\%$, while 30 (36.6%) had FAC $<35\%$. Conversely, ICU admission was more common in patients with reduced FAC, with 26 (68.4%) exhibiting FAC $<35\%$ compared to 12 (31.6%) with FAC $>35\%$. Regarding inotropic support, most patients (50, 65.8%) who did not require inotropes had FAC values $>35\%$, and those requiring inotropic support (30, 68.2%) predominantly had reduced FAC.

Patients requiring ventilatory support more frequently (18, 69.2%) had FAC $<35\%$ compared to those (8, 30.8%) with preserved FAC. A higher proportion (22, 57.9%) of patients developing renal deterioration had FAC values $<35\%$, although this association was not statistically significant. Prolonged

hospital stay of >7 days was more frequently (34, 56.7%) observed among patients with reduced FAC compared to those (26, 43.3%) with FAC $>35\%$. (Table-5).

Survivors ($36.48 \pm 4.12\%$) had a higher mean left ventricular ejection fraction compared to non-survivors ($34.21 \pm 3.17\%$), with the difference reaching borderline statistical significance. The mean tricuspid annular plane systolic excursion (17.95 ± 3.93 mm) was also greater among survivors than non-survivors (15.86 ± 3.35 mm), although this difference did not achieve statistical significance. Survivors demonstrated a higher mean right ventricular fractional area change ($35.24 \pm 6.68\%$) compared to non-survivors ($32.36 \pm 7.09\%$), without a statistically significant difference between the two groups. (Table-6).

Table-1: Demographic and baseline clinical characteristics of study participants (n=120)

Variable	Category	n (%)
Age	<60 years	87 (72.5)
	>60 years	33 (27.5)
Gender	Male	79 (65.8)
	Female	41 (34.2)
Hypertension (HTN)	Yes	98 (81.7)
	No	22 (18.3)
Diabetes Mellitus (DM)	Yes	31 (25.8)
	No	89 (74.2)
Ischemic Heart Disease (IHD)	Yes	44 (36.7)
	No	76 (63.3)
Chronic Kidney Disease (CKD)	Yes	14 (11.7)
	No	106 (88.3)

Table-2: Echocardiographic characteristics of study participants at admission (n=120)

Parameter	Mean $\pm$ SD/ n (%)
Left ventricular ejection fraction (%)	36.22 ± 4.07
TAPSE (mm)	17.71 ± 3.92
>16 mm	64 (53.3)
<16 mm	56 (46.7)
Right ventricular FAC (%)	34.90 ± 6.76
$>35\%$	64 (53.3)
$<35\%$	56 (46.7)
Pulmonary artery systolic pressure (PASP mmHg)	49.78 ± 7.64

Table-3: Short-term clinical outcomes of study participants during hospital Stay (n=120)

Outcome	Category	n (%)
In-hospital mortality	Yes	14 (11.7)
	No	106 (88.3)
ICU admission	Yes	38 (31.7)
	No	82 (68.3)
Inotropic support	Yes	44 (36.7)
	No	76 (63.3)
Mechanical ventilation	Yes	26 (21.7)
	No	94 (78.3)
Worsening renal function (WRF)	Yes	38 (31.7)
	No	82 (68.3)
Prolonged hospital stays (>7 days)	Yes	60 (50.0)
	No	60 (50.0)

Table-4: Association of tricuspid annular plane systolic excursion (TAPSE) with short-term clinical outcomes (n=120)

Outcome		TAPSE >16 mm n (%)	TAPSE <16 mm n (%)	p
ICU admission	No	52 (63.4)	30 (36.6)	0.001
	Yes	12 (31.6)	26 (68.4)	
Inotropic support	No	50 (65.8)	26 (34.2)	<0.001
	Yes	14 (31.8)	30 (68.2)	
Mechanical ventilation	No	56 (59.6)	38 (40.4)	0.009
	Yes	8 (30.8)	18 (69.2)	
Worsening renal function (WRF)	No	48 (58.5)	34 (41.5)	0.093
	Yes	16 (42.1)	22 (57.9)	

Table-5: Association of right ventricular fractional area change with short-term clinical outcomes [n (%)]

Outcome		FAC >35%	FAC <35%	p
ICU admission	No	52 (63.4)	30 (36.6)	0.001
	Yes	12 (31.6)	26 (68.4)	
Inotropic support	No	50 (65.8)	26 (34.2)	<0.001
	Yes	14 (31.8)	30 (68.2)	
Mechanical ventilation	No	56 (59.6)	38 (40.4)	0.009
	Yes	8 (30.8)	18 (69.2)	
Worsening renal function (WRF)	No	48 (58.5)	34 (41.5)	0.093
	Yes	16 (42.1)	22 (57.9)	
Prolonged hospital stay (>7 days)	No	38 (63.3)	22 (36.7)	0.028
	Yes	26 (43.3)	34 (56.7)	

Table-6: Comparison of echocardiographic parameters according to survival status (Mean±SD)

Parameter	Survivors (n=106)	Non-survivors (n=14)	p
LVEF (%)	36.48±4.12	34.21±3.17	0.050
TAPSE (mm)	17.95±3.93	15.86±3.35	0.060
FAC (%)	35.24±6.68	32.36±7.09	0.135

DISCUSSION

Traditionally, assessment and prognostication in heart failure have focused primarily on left ventricular systolic function. However, growing evidence indicates that right ventricular dysfunction plays a critical role in determining clinical outcomes, particularly during acute decompensation.¹¹ Right ventricular impairment contributes to systemic congestion, renal dysfunction, and poor response to therapy. Routine transthoracic echocardiography allows rapid bedside assessment of right ventricular function using simple parameters.¹² Tricuspid annular plane systolic excursion and right ventricular fractional area change are practical, reproducible indices that may help identify high-risk patients early during acute heart failure admissions.

The present study demonstrates that right ventricular dysfunction assessed by routine echocardiographic parameters is closely associated with adverse short-term outcomes in patients admitted with acute heart failure, and these findings are largely concordant with previously published international and regional literature. In our cohort, reduced TAPSE and reduced FAC were present in 46.7% of patients, indicating a high burden of right ventricular involvement during acute decompensation. Patients with

reduced TAPSE had significantly higher rates of ICU admission, inotropic support, and mechanical ventilation, while a similar pattern was observed for FAC, underscoring the clinical relevance of RV systolic impairment in the acute setting.

These findings align well with the study by Naseem *et al*¹³ who demonstrated that RV-pulmonary arterial uncoupling, assessed using the TAPSE/PASP ratio, was a strong independent predictor of in-hospital mortality in acute heart failure. Their reported univariate odds ratio and multivariable odds ratio emphasize the prognostic significance of RV dysfunction beyond conventional LV parameters. Although our study did not evaluate TAPSE/PASP as a composite index, reduced TAPSE alone was associated with a higher frequency of critical care needs, suggesting that even simple RV indices capture clinically meaningful haemodynamic compromise.¹³

The MRAHF cohort reported by Berrill *et al*¹⁴ demonstrated that TAPSE <17 mm and FAC <35% were associated with worse long-term outcomes. While their primary endpoints focused on 2-year mortality and major adverse events, the consistency of risk associated with guideline-defined TAPSE and FAC threshold support our observation that these parameters are clinically relevant even in short-term in-hospital phase. Moreover, their multivariable analysis showing RV FAC as an independent predictor reinforces the role of FAC as a robust marker of RV systolic performance.¹⁴

The association between RV dysfunction and mortality has also been demonstrated in non-AHF populations. Zornoff *et al*¹⁵ reported that in patients with LV dysfunction following myocardial infarction, each 5% reduction in RV FAC increased the odds of cardiovascular mortality by 16%. Although our study showed only borderline or non-significant differences in mean TAPSE and FAC between survivors and non-survivors, the numerical trends toward lower RV indices among non-survivors are directionally consistent with these findings and may reflect limited statistical power due to the relatively small number of deaths.

Importantly, our results regarding worsening renal function and length of stay are supported by the study of Genedi *et al*¹⁶ who reported a significant correlation between TAPSE<16 mm and adverse in-hospital outcomes, including WRF and prolonged hospitalization. In our cohort, both reduced TAPSE and reduced FAC were associated with higher proportions of WRF (although statistical significance was not consistently achieved) suggesting that venous congestion and impaired forward flow related to RV dysfunction may contribute to renal impairment during acute heart failure.

Our findings are consistent with those reported by Antit *et al*¹⁷ who demonstrated that reduced RV FAC was a strong predictor of adverse outcomes in acute

heart failure, with reported sensitivity of approximately 80% and specificity of 83% for composite poor outcomes. The study by Siddiqui *et al*¹⁸ evaluating right ventricular involvement in inferior myocardial infarction showed RV dysfunction in nearly half of patients using FAC and TAPSE, supporting the high prevalence of RV impairment observed in our cohort. Javed *et al*¹⁹ reported a significant positive correlation between TAPSE and right ventricular function aligning with our observation that reduced TAPSE was associated with increased ICU admission, inotropic support, and mechanical ventilation.

Evidence from regional and Pakistani studies further supports the prognostic importance of RV assessment. Khan *et al*²⁰ found that lower TAPSE values were associated with worse postoperative outcomes and longer hospital stay in patients undergoing rheumatic valve surgery, highlighting the impact of RV dysfunction on short-term recovery. Data from AFIC/NIHD and other local series have shown that impaired TAPSE and FAC correlate with in-hospital adverse events in conditions characterized by acute RV load, such as pulmonary embolism. Studies in myocardial infarction populations have also shown that RV involvement assessed by FAC and TAPSE is common and clinically meaningful, with significant correlations between TAPSE and RV function and a high prevalence of RV dysfunction ranging from approximately 40 to 50%. Brohi *et al*²¹ reported a high prevalence of RV dysfunction in ischemic heart disease patients, with significantly lower TAPSE values with RV dysfunction, reinforcing the link between RV impairment and disease severity.

STUDY LIMITATIONS

This study was conducted at a single centre, which may limit the generalizability of the findings. The sample size was relatively modest, and advanced echocardiographic techniques such as three-dimensional imaging or strain analysis were not evaluated. Long-term outcomes after hospital discharge were also not assessed.

CONCLUSION

Right ventricular dysfunction assessed by TAPSE and FAC on routine echocardiography is associated with adverse short-term clinical outcomes in patients with acute heart failure. These simple echocardiographic parameters provide valuable prognostic information at the time of admission. Incorporating routine ventricular assessment may improve early risk stratification and clinical decision-making in acute heart failure.

ACKNOWLEDGEMENT

We sincerely acknowledge the support and guidance of our mentors, colleagues, and the staff of the

participating hospital for their valuable assistance throughout this study.

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Received: 13 Mar 2026

Reviewed: 26 Mar 2026

Accepted: 31 Mar 2026

Contribution of Authors:

Authors approved the draft and are accountable in ensuring that questions related to accuracy or integrity of the work are duly investigated and resolved.

MM: Concept, study design, manuscript drafting and data analysis

SA: Data interpretation and supervision of study

SU: Assisted in study design, data collection and data interpretation

MA: Data collection, data entry and statistical analysis

Conflict of Interest: No conflict of interest is declared, **Funding:** None received from any agency



ORIGINAL ARTICLE

COMPARISON OF MULTIPLE ARTIFICIAL INTELLIGENCE-MODELS' PREDICTIVE POWER IN DETECTING DELAYED ANALGESIA IN EMERGENCY DEPARTMENT PATIENTS

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Background: Artificial intelligence (AI) has significant potential to enhance risk assessment by identifying patients at higher risk of delayed analgesia. The goal of this work was to create and validate AI models that predict the probability of delayed analgesia, and compare the predictive power of multiple AI models in detecting delayed analgesia in emergency department (ED) patients and avoiding longer patient stays. **Methods:** From Dec 2024 to Jan 2025, 300 adult patients with moderate to severe pain were studied in the Emergency Department of an academic facility teaching hospital. This retrospective observational study was collected and analysed retrospectively, with age, gender, triage category, triage pain score, and presentation during peak hours serving as input features. Five machine learning models were constructed and compared for their accuracy to forecast delayed analgesia. Important predictors were identified using SHAP (SHapley Additive exPlanations) values for the AI model with the highest accuracy. **Results:** Random Forest and J48 achieved 77% accuracy, with Random Forest having greater recall for delayed cases (Precision=0.71, Recall=0.84) for anticipating delayed analgesia. Naive Bayes and Logistic Regression had low recall for delayed cases, while MLP Neural Network demonstrated moderate predictive usefulness. Random Forest model had the best performance with the highest AUC [0.83 (95% CI: 0.75–0.90)] on ROC analysis. **Conclusion:** AI models were successfully implemented, Random Forest outperforming the others, for early identification of patients at risk of delayed analgesia. AI models can decrease unnecessary delays and improve pain management in ED.

Keywords: Acute pain management, Artificial Intelligence, Delayed analgesia, Emergency medicine, Machine Learning, Random Forest, Triage

Pak J Physiol 2026;22(1):52–7, DOI: <https://doi.org/10.69656/pjp.v22i1.1931>
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INTRODUCTION

Delayed analgesia is generally defined as the time to get pain medication for patients presenting with painful conditions since their arrival in the emergency department. There is no single universal 'standard' definition for it, as the specific time frame can vary depending on the clinical context and institutional guidelines. The Royal College of Emergency Medicine advises giving adequate analgesia to patients arriving with moderate to severe pain within 15 minutes of presentation.¹ Often used as a quality indicator of patient care, emergency departments around the world struggle with the problem of delayed analgesia. More than a major percentage of patients experience delays longer than the first hour of ED visit, with a median time to analgesia exceeding two hours.^{2,3} Lengthy hospital stays create lower patient satisfaction, and inadequacy of pain management is all linked to these delays.

Traditional triage methods have a reduced dynamic capability to identify individuals at risk of under treatment. The intricate correlation between patient demography, pain intensity, departmental workload, and understaffing in emergency department settings may not be sufficiently captured by traditional triage systems, which depend more on rule-based

algorithms and clinician judgment. Because of this, patients with similar levels of pain may have quite diverse analgesic timeframes.

Machine learning (ML), a subset of AI, provides a clear benefit in emergency treatment by providing real-time risk stratification using routinely obtained triage data. The ML algorithms, in contrast to traditional statistical models, are capable of integrating high-dimensional data, modelling non-linear relationships, and adapting to varied patient strata. Because of these characteristics, machine learning is especially well-suited for dynamic yet time-sensitive environments such as the Emergency Department (ED), where quick, data-driven decisions may help enhance rather than replace clinical judgment.^{4–7}

Literature supports the use of ML in acute care and pain management. AI-driven models have shown improved real-time pain management after caesarean delivery, better prediction of analgesic needs in postoperative populations, and precise identification of patients at risk for long-term opiate usage.^{6,8–9} Explainability in AI is a crucial prerequisite for clinician trust, ethical use, and system acceptance of AI in clinical practice. Explainable AI, such as SHAP provides transparent, case-level, and

population-level insights into model predictions. Explainable ML enhances clinical interpretability, facilitates auditability, and predicts potential bias by determining the relative contribution of individual input variables.¹⁰ These important factors are highlighted in the STROBE-AI and JMIR-AI reporting standards. The use of explainable ML models to forecast delayed analgesia at the triage level is mainly unexplored. Bridging this gap could help identify high-risk patients early, promote focused care, and provide guidance for quality improvement initiatives.

This study aimed to develop and validate explainable AI models to predict delayed analgesia among ED patients using triage data. The secondary objective was to compare the predictive performance of five machine learning algorithms—Random Forest, Multi-layer Perceptron (MLP), J48 Decision Tree, Naive Bayes, and Logistic Regression, with reference to delayed analgesia.

METHODOLOGY

A total of 300 patients' data were analysed in a retrospective analytical study from emergency medical records. Data of one patient was deleted who had mild pain (pain score 3/10). Random selection was used to include patients (≥ 18 years old) with moderate to severe pain (Numeric Rating Scale $\geq 4/10$) in the emergency department of a teaching hospital, which receives more than 80,000 ED visits annually. Patients who were triaged for emergency resuscitation or had missing values were excluded from the final sample by list-wise deletion.

The sample size was determined based on the stated accuracy (72%) of the reference AI model⁵ with a confidence interval of 95%, a study power of 80%, and a $\pm 5\%$ margin of error. Our study included 300 patients, a number close to the calculated size ($n=310$) and deemed adequate. As per the literature review, no AI model had predicted delayed analgesia in emergency rooms previously; the authors relied on a post-operative patient study⁵ for sample computation.

Input variables (age, gender, initial pain score, triage category, and presentation during peak hours) were documented at triage, and the time to analgesia was reported at the patient's bedside. Features were selected while considering their influence over the promptness of acute pain management in prior literature, such as pain severity¹¹, triage level¹², overcrowding¹³, along with patient demographics¹⁴. The pain score (4–10 for moderate to severe) was reported using the Numeric Rating Scale (NRS), a freely available, frequently used 11-point pain assessment tool that rates pain on a scale of 0 to 10 (0 as no pain, 1–3 labelled as mild, 4–6 as moderate, and 7–10 as severe pain out of 10).¹⁵ The

triage category had been assigned according to the Emergency Severity Index (ESI, Version 4), a validated, five-level triage method (from Level 1= most urgent, e.g., unresponsive, to Level 5= least urgent, e.g., history/exam only), freely available for non-commercial research and clinical use.^{16,17} Trained staff were deployed for triaging, and the senior triage person was responsible for double-checking the category to avoid personal bias. Presentation during peak hours was documented as nominal data. Time to analgesia of more than 15 minutes since arrival was defined as delayed analgesia, and it was reported as a primary outcome.¹

The data file of 299 patients in SPSS-25 was exported to Python 3 (Colab) for building and validating AI models. Continuous variables were reported as Mean $\pm$ SD, while skewed data distributions were represented as medians (IQR). Categorical variables were reported as frequencies with proportions. All tests were two-tailed with a significance level of $p < 0.05$. Statistical analysis was done on IBM SPSS-25.¹⁸ AI/ML models were created in Python (Version 3.8) using the Google Colaboratory environment,^{19,20} both of which are freely available for research and academic purposes. Data was cleaned by removing invalid or missing values, numerically encoding binary and categorical characteristics, and standardizing input data formats. Categorical variables were transformed using one-hot encoding. To address class imbalance, the Synthetic Minority Over sampling Technique (SMOTE)²¹ was applied resulting in a balanced training set of records. Feature scaling was applied only to the input of the Multi-Layer Perceptron (MLP) model.

Five supervised machine learning algorithms were trained using Python's scikit-learn²² and imbalanced-learn libraries²³ and a fixed random state for reproducibility: Random Forest, J48 (decision tree), Logistic Regression, MLP, and Naive Bayes. Data was split using a 70/30 stratified split for training and testing. Models were evaluated on the holdout test set. The area under the receiver operating characteristic curve (AUC/ROC) was computed for all models to assess the individual model's discriminative power.

To reflect performance on clinically significant instances, class-wise metrics such as accuracy, precision, recall, F1-score, confusion matrices, macro, and weighted averages were explicitly examined for class 1 (delayed analgesia) and class 0 (timely analgesia). SHAP analysis was performed to identify the input variables with the highest prediction probabilities.²⁴

Ethical approval for this study was obtained from the Institutional Review Board/Ethical Review Board, which waived consent for the study. The study workflow is shown in Figure-1.

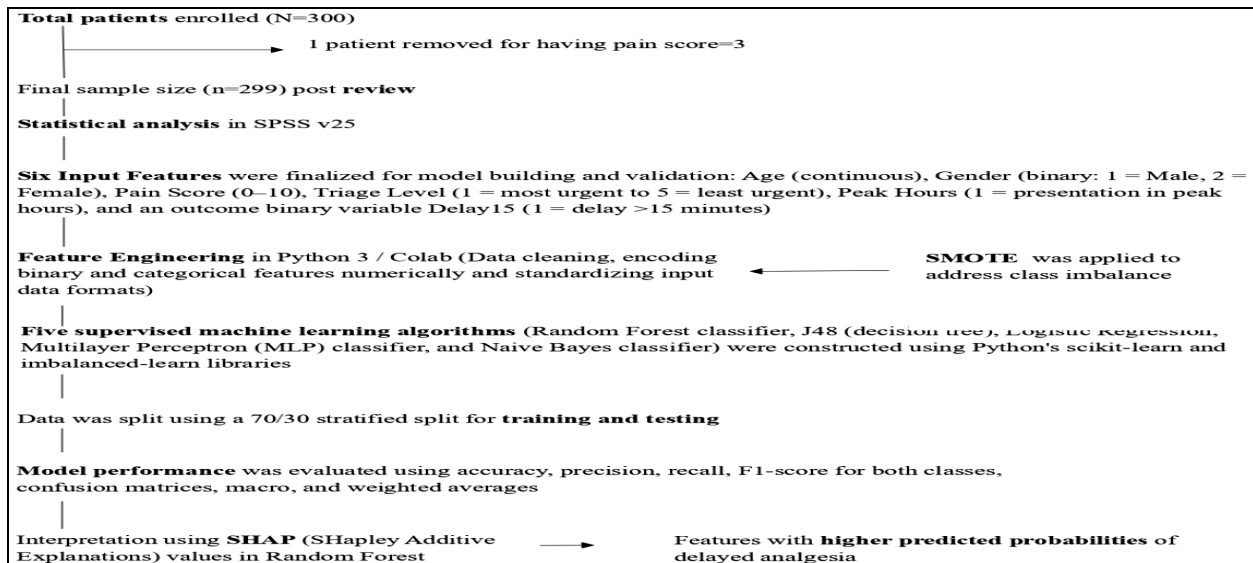


Figure-1: The study workflow

RESULTS

The study involved 299 individuals, with 24.7% experiencing delayed analgesia. The cohort's median age was 38 years (IQR 21), with a male predominance, and no significant difference in age between the two groups ($p=0.341$). Severe pain was reported by most of the patients, with similar rates in both groups (82.4% vs 81.3%, $p=0.899$). The majority of patients (68.2%) reported during peak hours. Critical triage was allocated to 45.5% of the total cohort, with similar distributions between the delayed and timely groups (40.5% vs 47.1%, $p=0.325$). The delayed group had slightly higher mean age, larger male preponderance, and a higher proportion of presentations during peak hours than the timely group, but pain scores and triage distributions were similar across both groups (Table-1).

The confusion matrix analysis added to our understanding of each model's classification behaviour and offered information on its classification performance. Random Forest properly recognized 55 true positives and 49 true negatives, with 12 false positives and 19 false negatives, demonstrating reliable discrimination between the two classes. J48 Decision Tree performed similarly, with more false negatives ($n=17$). While Naive Bayes had the highest number of true positives (61), it also had the highest number of false positives ($n=26$) resulting in lower overall specificity. In contrast, MLP and Logistic Regression provided more balanced but less accurate outputs, with more false negatives (24 and 31, respectively) which may restrict their clinical reliability.

The ROC curves for all five models revealed that all classifiers outperformed chance (diagonal reference line, AUC=0.5), (Figure-2). The Random Forest model consistently demonstrated the greatest

divergence from the reference line, particularly in the higher sensitivity range, resulting in the best AUC (0.83). The J48 decision tree (AUC=0.80) and MLP (AUC=0.79) both performed well, with ROC curves trailing the Random Forest. Naive Bayes (AUC=0.76) demonstrated moderate separation, whereas logistic regression (AUC=0.72) had the lowest curve elevation, indicating a lesser discriminative capacity to identify complicated predictor associations.

The Random Forest classifier demonstrated the best overall performance, with an accuracy of 77% (95% CI: 69–84), an F1 score of 0.77 for delayed instances, and an AUC of 0.83 (95% CI: 0.75–0.90), indicating a balanced sensitivity and specificity. The J48 Decision Tree similarly demonstrated significant predictive abilities with slightly higher precision for non-delayed cases than Random Forest, but marginally lower recall for delayed cases. In comparison, the Naive Bayes model performed less well, primarily due to a lower recall for delayed cases, despite achieving adequate precision. This result shows that Naive Bayes underperformed in detecting patients at risk of delayed analgesia, which is crucial in a therapeutic setting. The MLP demonstrated moderate predictive performance, with balanced but modest precision and recall. Finally, logistic regression showed the lowest predictive accuracy and AUC, with a lower recall for delayed cases (Table-2).

SHAP analysis on the top-performing AI model (Random Forests) revealed the mean absolute SHAP values of age (0.074568) and pain score (0.074568) as the two most significant parameters influencing the model's output (Figure-3). A higher score for these features indicated a greater probability of a delay, suggesting that older patients with higher pain scores are more likely to experience delayed pain alleviation.

Table-1: Baseline characteristics of study cohort and stratified comparison of delayed and timely analgesia [n (%)]

Characteristics	Overall (n=299)	Delayed Analgesia (n=74, 24.7%)	Timely Analgesia (n=225, 75.3%)	<i>p</i>
Age (Years) [Median (IQR)]	38 (21)	38 (21.5)	38 (21)	0.341
Gender				
Male	218 (72.9)	58 (78.4)	159 (70.7)	0.197
Female	81 (27.1)	16 (21.6)	66 (29.3)	
Pain Score				
Severe	245 (81.9)	61 (82.4)	183 (81.34)	0.899
Moderate	54 (18.1)	13 (17.56)	42 (18.67)	
Presentation Time				
Peak	204 (68.2)	45 (60.8)	158 (70.2)	0.133
Non-peak	95 (31.8)	29 (39.2)	67 (29.8)	
Triage Level				
Critical	136 (45.5)	30 (40.5)	106 (47.1)	0.325
Non-critical	163 (54.5)	44 (59.5)	119 (52.9)	

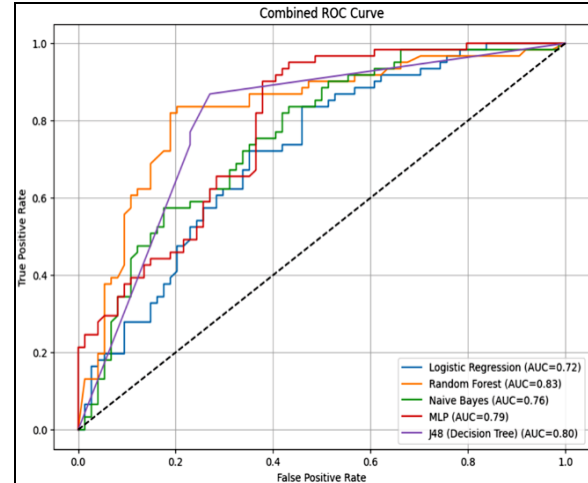


Figure-2: Combined receiver operating characteristic (ROC) curves for five machine learning models

Table-2: Comparison of the predictive performance of AI models

Models	Accuracy (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	AUC/ROC (95% CI)
Random Forest	77 (69–84)	74.3 (63–83)	80.3 (69–89)	82 (71–90)	72 (60–82)	0.83 (0.75–0.90)
J48 Decision Tree	77 (69–84)	77.0 (66–86)	77.0 (65–86)	80.2 (69–89)	73.4 (61–83)	0.80 (0.72–0.88)
Naive Bayes	71 (63–79)	82.4 (72–90)	57.3 (45–69)	70.1 (59–80)	72.9 (59–83)	0.76 (0.67–0.84)
MLP	67 (59–75)	67.5 (55–78)	67.2 (55–78)	71.4 (59–82)	63 (51–74)	0.79 (0.70–0.87)
Logistic Regression	64 (55–72)	60.5 (48–72)	72.1 (60–82)	71.6 (59–82)	58.6 (46–71)	0.72 (0.63–0.81)

PPV=Positive predictive value, NPV=Negative predictive value

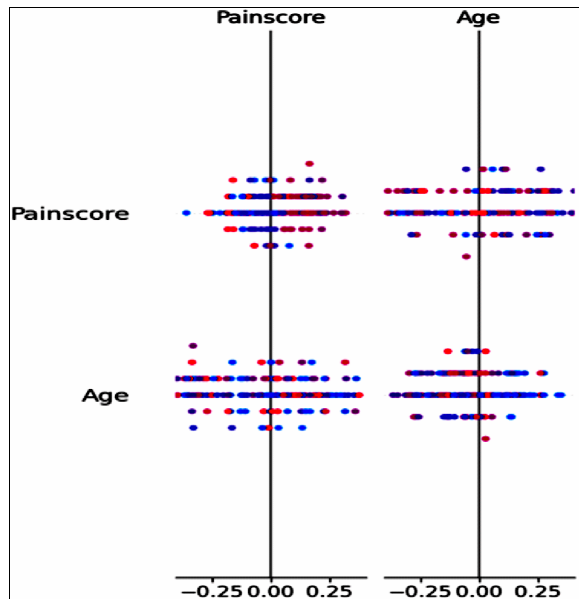


Figure-3: Beeswarm plot for SHAP analysis of top predictors

DISCUSSION

Predicting delayed analgesia entails identifying high-risk individuals based on clinical criteria. While AI is working in medical predictive and risk stratification domains, this study is among the initial ones to demonstrate its efficacy in predicting delayed analgesia in the emergency departments using quick

triage data, —a previously unexplored area. The study employed machine learning techniques, including Random Forest, Naive Bayes, Multilayer Perceptron, J48 Decision Tree, and Logistic Regression, to predict delayed analgesia delivery in emergency department patients by examining complex interactions.

The comparative analysis of the models suggested that ensemble and decision-tree models (Random Forest, J48) handled structured triage data precisely, demonstrating their clinical utility in predicting delayed analgesia in the emergency department. The Random Forest model had the greatest AUC and balanced classification metrics. It was able to identify both delayed and non-delayed analgesia cases at a rate of 77% (the highest predictive accuracy among all five models). From a clinical standpoint, Random Forest and J48 provided the most dependable trade-off between sensitivity and specificity, ensuring the identification of at-risk individuals without over classification. Previous literature has also supported the efficacy of Random Forest in a systematic review of fifteen studies, with the decision-tree-based boosting algorithms in 5 studies with AUC ranging from 0.81 to 0.66 as the best performing models.⁹

SHAP analysis on the Random Forest revealed that older patients with higher pain scores were more likely to experience delayed pain relief. Bloom *et al*²⁵ and Platts-Mills *et al*²⁶ have also made

similar observations, supporting the idea that SHAP-informed AI tools can help risk classification at ED triage and provide a more comprehensive understanding of the model.

A fundamental strength of this study is the use of accessible triage-level data, which renders the models as simple, explainable, and viable for real-time deployment in high-pressure emergency departments. Gabriel *et al*⁴ created a neural network model to predict outpatient opioid refills after surgery, with an AUC of 0.75 and an accuracy of 73%, and our study, which relied entirely on triage-stage information, achieved a higher accuracy and AUC while producing fewer false negatives. Similarly, other studies with comparable model accuracy have found promising results in management pathways.^{5,27} Our methodology was generalized across varied emergency patients and explicitly favoured strong recall (84%) to reduce analgesic delays—a critical goal in emergency medicine.

LIMITATIONS

The limited sample size of this study makes generalizability difficult, despite the use of cross-validation processes. The models were verified internally on a single-centre basis and excluded ED operational features such as staff-to-patient ratios, revealing departmental work intensity. These factors demonstrated the challenges of assessing time-to-analgesia in real-world emergency rooms.

CONCLUSION

The study shows the successful implementation of AI models to predict delayed analgesia in emergency department patients using easily accessible triage data. Our approach provides a preliminary, interpretable, and practical solution for early identification of patients at risk of delayed analgesia. Random Forest outperformed the other AI models in predicting delayed analgesia. However, external validation is recommended, and AI models can be integrated into clinical decision support systems to optimize patient care pathways, decrease unnecessary delays, and improve pain management in high-volume emergency settings.

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Received: 27 Jan 2026

Reviewed: 20 Mar 2026

Accepted: 31 Mar 2026

Contribution of Authors:

Authors approved the draft and are accountable in ensuring that questions related to accuracy or integrity of the work are duly investigated and resolved.

AS: Conceptualization, drafting initial manuscript, final proofreading

MMAB: Ideation, first drafting, final proofreading

ZR: Ideation, first drafting, final proofreading

ZS: Development and design of AI models, proof reading

Conflict of Interest: No conflict of interest is declared,	Funding: None received from any agency
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ORIGINAL ARTICLE

RENAL FUNCTION AND OXIDATIVE STRESS IN DOXORUBICIN-TREATED WISTAR RATS: IMPLICATIONS FOR NEPHROTOXICITY

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Background: Doxorubicin is the most potent broad-spectrum anthracycline antibiotic. Despite having increased anti-tumour activity, it has been reported toxic to different organs including kidneys. This study was conducted to investigate nephrotoxic effects of Doxorubicin by assessing the renal functions parameters and oxidative stress in adult male Wistar rats. **Methods:** From September 2023 to February 2024, this quasi-experimental study was carried out in Department of Pharmacology, Isra University, Hyderabad. Twenty healthy male rats, weighing 180–220 grams, were divided into Group I (control) which received 10 mL distilled water I.P., and Group II (experimental) which received injections of doxorubicin (1.2 mg/Kg body weight) I.P. twice a week for 21 days. The animals were slaughtered; blood samples were taken for renal function tests, and oxidative stress markers. Histological analyses were carried out for nephrotoxicity. **Results:** Group II rats showed a significant decline in absolute kidney weight ($p<0.05$) accompanied by a significant decrease in animal weight. These rats also exhibited significantly higher ($p<0.05$) serum levels of renal and inflammatory indicators. Serum levels of superoxide dismutase, reduced glutathione, glutathione peroxidase and catalase in renal tissues were statistically substantially lower ($p<0.05$) in Group II, but malondialdehyde and Nitric oxide levels were significantly higher ($p<0.05$) in the same group. Histopathological analysis revealed renal tubule dilation and reduced number of renal corpuscles in Group II rats. **Conclusion:** Doxorubicin poses a significant damage to the renal tissues by imbalance between antioxidant and free oxygen radicals, which causes protein oxidation resulting in tissue damage.

Keywords: Doxorubicin, Histopathology, Nephrotoxicity

Pak J Physiol 2026;22(1):58–61, DOI: <https://doi.org/10.69656/pjp.v22i1.1889>

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INTRODUCTION

Kidneys are essential for homeostasis as they remove toxic substances from metabolism and control fluid volume and electrolyte balance.¹ Kidney injuries, whether acute or chronic, arise from various factors and are recognized as a significant public health issue. Recent studies show that chronic kidney disease (CKD) affects approximately 10% to 14% of the general population worldwide. The total burden of kidney related diseases has nearly doubled during the last several decades. Over 850 million individuals are suffering from various kidney related diseases. The disease creates a bigger problem for specific high-risk groups because about 33% of diabetic patients and 20% of hypertensive patients develop CKD which now ranks as the 9th most common cause of death world-wide.²

Several factors contribute to renal injury, including external chemicals, such as medication like doxorubicin (DXR), that have the potential to harm renal tissue. The kidney damage resulting from DXR shows itself through an acute decline in kidney function. This damage is not limited to sudden injury; the condition usually develops into a chronically damaged

renal parenchyma. Whether the injury is acute or chronic, the underlying nephrotoxic mechanism eventually impairs the filtration process, resulting in accumulation of metabolic products such as creatinine and urea in blood.³

Doxorubicin is the most powerful and effective broad-spectrum anthracycline antibiotic. It has been used as an anti-cancer medication since 1960s to treat lymphomas and a wide range of solid cancers.³ Despite having increased anti-tumour activity, DXR's toxicity to the heart, kidneys, lungs, testicles, and blood has limited its usage in chemotherapy. DXR is reported to work by obstructing the synthesis of macromolecules, inhibiting topoisomerase II, halting the growth of tumour cells in the G2 phase, causing apoptosis.^{4,5} DXR creates oxidative stress through its ability to disrupt the balance between free radicals and antioxidants which leads to lipid peroxidation that damages tissue structures. Although the exact process remains unclear it generates an imbalance between free radicals and antioxidants, which leads to lipid peroxidation (LPO) macromolecules and iron-based damage of cell membranes and large molecular structures.^{6,7} This study aimed to investigate the DXR-induced nephrotoxic

effects in adult Wistar rats by analysing renal biomarkers and oxidative damage of DXR by assessing the renal functions parameters and oxidative stress.

MATERIAL AND METHODS

This quasi-experimental study was done in the Department of Pharmacology, Isra University, Hyderabad from Sep 2023 to Feb 2024 after approval from the Ethical Review Committee of Isra University (IU/RR-10-ERC-23/N/2023/295). In accordance with international guidelines for the care and use of laboratory animals and institutional regulations, the study involved 20 healthy adult male Wistar rats, aged 9–12 weeks and weighing 180–220 grams, with no visible deformities or abnormalities. The sample size was calculated to be 20, using G*Power 3.1 assuming $\alpha=0.05$ and a large effect size ($f>0.60$), which is typical for DXR-induced organ injury.

The animals were kept in clean, well-ventilated cages with unrestricted access to balanced laboratory diet and water, with 12-hour light-dark cycle at a temperature of $22\pm 2^\circ\text{C}$ for one week for acclimatization. The body weights of all animals were recorded, and they were randomly assigned into two equal groups ($n=10$).

Group I was labelled as control and group II as experimental. Group I animals received 1 mL distilled water intra-peritoneal for 21 days, and normal chow (twice a week), while group II rats received DXR intra-peritoneal at 1.2 mg/Kg body weight (twice a week) for 21 days, and normal chow.⁸ After 4 hours of administration of last dose, all rats were weighed again.

The animals were subsequently sacrificed under anaesthesia with sodium pentobarbital (45 mg/Kg) and dissected. Blood samples were collected through cardiac puncture to assess serum creatinine, blood urea nitrogen (BUN), and C-reactive protein (CRP) levels. Malondialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione (GSH), glutathione peroxidase (GPx), and nitric oxide (NO) were also assessed as markers for oxidative stress. All tests were performed on Roche/Hitachi diagnostic kits, whereas GPx was performed on the bioassay technology ELISA kit. The kidneys of all animals were removed immediately, weighed, and examined grossly for any morphological changes. The Relative Tissue Weight Index (RTWI) was calculated by employing following formula:⁴

$$RTWI = \frac{\text{Paired weight (g) of kidneys}}{\text{Animal body weight (g)}} \times 100$$

The kidneys were sliced into small pieces of 3 mm and fixed in 10% neutral formalin for 48 hours. The tissue slices were processed for sectioning at a

thickness of 4 μm . The sections were stained with Haematoxylin and Eosin (H&E) and examined under a light microscope at 100 magnification. Quantitative measurements, renal corpuscular diameter, and appearance of proximal and distal convoluted tubules were assessed using a stage micrometer. The qualitative parameters including vacuolization within PCT and DCT, glomerular and stromal vascular congestion, inflammatory cells infiltration were observed.

Data was analysed using SPSS-24. Qualitative variables were presented as frequency and percentage while Mean $\pm$ SD was employed for quantitative variables. Student's *t*-test was used for analysis of quantitative data, and $p\leq 0.05$ was considered as statistically significant.

RESULTS

Table-1 shows the mean body weights (pre- and post-experimental) along with kidney weight and relative tissue weight index of both group rats. A significant decline in body weight, kidney weight and RTWI ($p<0.05$) was observed in group II rats. (Table-1).

Table-2 is demonstrating the comparison of renal markers, i.e., serum creatinine and BUN, and inflammatory marker (CRP levels) between control and DXR treated group. A statistically significant rise ($p<0.05$) in S. creatinine, BUN and CRP concentrations were observed in group II rats treated with DXR alone compared with group I controls.

DXR induced group II showed the oxidative stress with significantly ($p<0.05$) lowered levels of GPx, GSH, SOD and catalase in renal tissues, while MDA and NO levels were significantly higher ($p<0.05$) compared with the control group. (Table-3).

Table-1: Body weight (pre- and post-), kidney and relative tissue weight index of both study groups

	Group I	Group II	<i>p</i>
Pre-body weight (g)	188.3 $\pm$ 7.81	191.5 $\pm$ 8.11	0.381
Post-body weight (g)	192.2 $\pm$ 6.30	173.2 $\pm$ 5.81	0.000
Kidney weight (g)	1.69 $\pm$ 0.07	1.41 $\pm$ 0.04	0.000
RTWI (%)	0.88 $\pm$ 0.04	0.82 $\pm$ 0.03	0.001

Table-2: Comparison of serum renal markers and CRP levels between both groups

	Group I	Group II	<i>p</i>
S. Creatinine (mg/dL)	0.43 $\pm$ 0.03	0.79 $\pm$ 0.08	0.000
BUN (mg/dL)	22.41 $\pm$ 2.50	51.20 $\pm$ 2.91	0.000
C-Reactive Protein (mg/dL)	0.11 $\pm$ 0.09	0.83 $\pm$ 0.31	0.000

Table-3: Comparison of oxidative stress markers between both groups

	Group I	Group II	<i>p</i>
GPx (U/mg protein)	9.89 $\pm$ 1.30	6.78 $\pm$ 0.71	0.000
GSH ($\mu\text{mol/g}$ tissue)	1.16 $\pm$ 0.01	0.31 $\pm$ 0.02	0.000
SOD (U/mg protein)	13.17 $\pm$ 0.61	10.37 $\pm$ 0.91	0.000
Catalase ($\mu\text{mol/g}$ protein)	19.38 $\pm$ 1.1	15.67 $\pm$ 1.4	0.000
MDA (nmol/mg/protein)	0.71 $\pm$ 0.05	2.18 $\pm$ 0.18	0.000
NO ($\mu\text{mol/g}$ /protein)	0.13 $\pm$ 0.02	0.63 $\pm$ 0.01	0.000

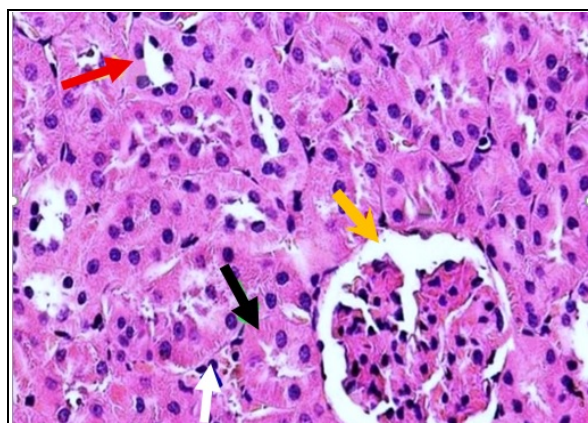


Figure-1a: (Group I) Normal glomerular structure is seen. Renal corpuscle (Yellow arrow). Proximal convoluted tubules (Black arrow). Distal convoluted tubules (Red arrow). Stroma (White arrow)

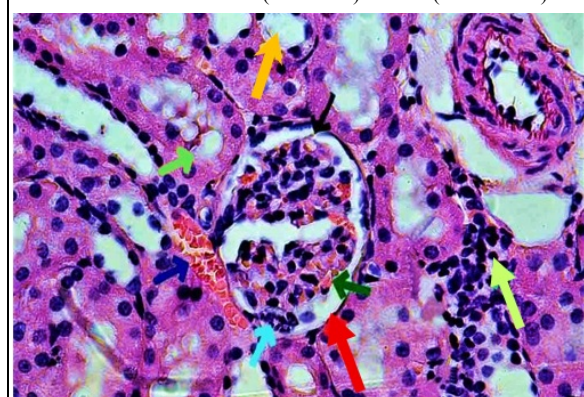


Figure-1b: (Group II) Glomerular atrophy and fibrosis is seen. Renal corpuscle (red arrow) with glomerulus congestion (dark green arrow) and inflammatory cell infiltration (aqua blue arrow). Cytoplasmic vacuolization in PCT (light green arrow) and DCT (yellow arrow). Stromal congestion (blue arrow) and inflammatory cell infiltration (aqua green arrow)

Figure-1a & 1b: Photomicrograph of renal tissue of control and DXR treated groups

The cytoplasmic vacuolization in PCT and DCT were observed along with the vascular congestion of glomerulus and stroma as well as infiltration of inflammatory cells was demonstrated in group II renal tissues compared with group I tissues. There was statistically significant ($p < 0.05$) decline in renal corpuscles diameter of group II ($73.1 \pm 3.0 \mu\text{m}$) compared with group I ($84.8 \pm 2.9 \mu\text{m}$). There was a significant ($p < 0.05$) increase in diameter of PCT and DCT of group II ($45.5 \pm 3.7 \mu\text{m}$ and $41.3 \pm 5.2 \mu\text{m}$) compared with group I ($33.7 \pm 4.2 \mu\text{m}$ and $32.5 \pm 2.9 \mu\text{m}$) respectively.

DISCUSSION

DXR is an effective treatment for various solid tumours; however, it can significantly impair kidney function, similar to other anti-tumour medications. DXR accumulates in the glomerulus, leading to substantial kidney damage. The mechanisms through which DXR

causes glomerular toxicity remain poorly understood.^{9,10}

This study discovered substantial alteration following DXR administration, which disrupted the BUN, Creatinine, and markedly affected renal histological damage. A comparison of mean animal post-experimental body weight with mean pre-experimental body weight in both groups showed a significant difference. A gradual normal increase in body weight in the control group I was observed. Statistically significant weight loss in DXR treated group II ($p < 0.05$) was noted. Similar results were demonstrated by Munawar *et al*⁸ and Chen *et al*¹¹ reported that rats had significant decline in body weight after induction with DXR compared with the control group.

A significant decrease ($p < 0.05$) in mean absolute kidney weight and RTWI of rats treated with DXR compared to the control group was observed. Consistent with the results from the studies by Chen *et al*¹¹ Sami *et al*¹² and Khan *et al*¹³ the current study demonstrated a significant decrease in kidney weight and RTWI following DXR administration. This may be due to adverse effects of DXR causing the atrophic and degenerative changes resulting in damage to the kidneys.

In our study, we showed that DXR treatment led to a substantial increase in MDA levels, accompanied by significant decreases in GSH content and the activities of GPx and SOD. These results align with findings reported by Liu *et al*⁴ and Khan *et al*¹³.

Histopathological analysis of renal sections of both group rats was done in this study to demonstrate the impact of DXR on renal tissues compared with the control rats. It has been observed that DXR treated rats for 21 days had shown significant atrophic changes in the glomerulus including many shrunk renal corpuscles and degenerated renal tubules (PCT and DCT) with decreased diameter as well having disrupted basement membrane, discontinuous brush border of PCT, stroma of the kidney appeared vacuolated with focal haemorrhages, and inflammatory cells infiltrate was present. In addition, blood vessels were congested. A significant decrease in the diameter of the renal corpuscle due to glomerular degeneration and vacuolation in the DXR treated group. Chen *et al*¹⁰ and Afsar *et al*¹⁴ also reported the similar findings which they have demonstrated under microscopic examination in their studies.

In the current study, renal sections of rats treated with DXR 1.2 mg/Kg twice a week for 21 days showed statistically significant histological changes including the increase in diameter of PCT and diameter of DCT with degenerated cells in DXR treated group II was analogous to a study done by, Liu *et al*⁴, Munawar *et al*⁸ and Sami *et al*¹², who assigned renal pathologies due to production of oxygen derived free radicals and

reactive oxygen species (ROS) leading to oxidative damage. This may be due to the fact that oxidative stress resulting in the free radicals resulting from the DXR induction. These substances are harmful to biological systems as they react with protein, DNA and lipids causing cellular damage. Moreover, vacuolization within cells of PCT and DCT might be the signs of renal toxicity and cell degeneration in DXR treated group II. These findings are supported by those observed by Al-Karawi *et al*¹⁴ Al-Karawi *et al*¹⁵.

Furthermore, our histopathological findings also revealed the congestion of blood vessels with stagnant blood cells & disrupted endothelium causing haemorrhage within renal stroma in DXR treated group II. This may be due to the prevention of prostaglandin synthesis which could have regulated blood flow. Stromal inflammatory cell infiltrate in the present work was mainly the lymphocytes (mononuclear leukocytes) in DXR treated group. The migration of leucocytes towards the inflammatory site is called chemotaxis which is response of body tissue facing any injurious impact which were also reported by Afsar *et al*, and Al-Karawi *et al*, in their studies.^{14,15}

With strengths there are limitations in this study as the present student observed only DXR toxic effects on renal system and not on other organ system due to lack of funding and time constraints. Moreover, no high or low dose was used to compare the dose effects in this study.

CONCLUSION

DXR induces a significant renal tissue injury by precipitating an imbalance between antioxidant defenses and free oxygen radicals. This oxidative stress leads to protein oxidation, ultimately resulting in progressive tissue damage.

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Received: 11 Aug 2025

Reviewed: 15 Feb 2026

Accepted: 24 Feb 2026

Contribution of Authors:

Authors approved the draft and are accountable in ensuring that questions related to accuracy or integrity of the work are duly investigated and resolved.

FR: Concept, Data Collection, Drafting, Writing and Final Revision

SK: Literature Search, Data Collection, Statistical analysis

AA: Data Collection, Statistical analysis

SBM: Concept, Drafting, Writing and Revision

AK: Concept, Literature Search, Statistical Analysis

RQ: Data Collection, Drafting of Manuscript

Conflict of Interest: No conflict of interest is declared, **Funding:** No funding received from any agency



REVIEW ARTICLE

FROM BITE TO BRAIN: A DEEP DIVE INTO THE RABIES VIRUS AND ITS IMPACTS

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Rabies lyssavirus is an enveloped, neurotropic virus with a single-stranded, negative sense RNA genome of approximately 12 kilobases, and is responsible for rabies in animals and humans. Rabies virus (RABV) displays a bullet-shaped morphology and has a broad host range. Rabies is severe, often life-threatening disease, endemic in many regions of Africa and Asia, with cases frequently under reported due to limited laboratory diagnostics. Transmission primarily occurs via the infected animal's saliva, especially dogs, which account for over 99% of human rabies cases. Following exposure, the virus initially infects peripheral motor neurons and subsequently invades the central nervous system (CNS), leading to fatal outcomes once symptoms develop. Prevention relies on mass dog vaccination, prompt post-exposure prophylaxis, and thorough wound care. Administration of rabies vaccine and immunoglobulin is essential for effective prevention. Achieving the World Health Organization's (WHO) target of eliminating dog-mediated human rabies by 2030 depends on strong government support, targeted vaccination, and increased public awareness.

Keywords: Endemic, Infected animal, Neurotropic, Pathogenesis, Rabies virus

Pak J Physiol 2026;22(1):62–7, DOI: <https://doi.org/10.69656/pjp.v22i1.1858>

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INTRODUCTION

Rabies is an extremely fatal viral disease that evokes significant fear due to its severe neurological sign and symptoms and near-certain mortality once symptoms appear. Caused by a member of the lyssavirus genus, rabies primarily affects animals, with human cases being relatively rare.¹ Saliva of the infected animals is the main transmitter of rabies that typically occurs through bites or scratches.

After entering the body, rabies virus spreads to peripheral nerves and then to the CNS, where it causes the inflammation of the brain and the spinal cord.² The incubation period typically ranges from several weeks to months, often delaying diagnosis, and initial symptoms resemble a flu-like condition before progressing to anxiety, hallucinations, hydrophobia, and ultimately coma, and death.³

Effective prevention remains the cornerstone of rabies control. Immunizing domestic animals such as cats and dogs, practicing good hygiene principles as pet owners is necessary to reducing transmission risk.⁴ For individuals exposed to rabid animals, prompt administration of post-exposure prophylaxis (PEP), including vaccination and immunoglobulin is vital.⁵ Despite advances in surveillance and prevention, rabies persists in areas with inadequate veterinary and healthcare infrastructure. This article will explore the symptoms, diagnosis, treatment, and prevention strategies for rabies.

HISTORY

Rabies, is one of the earliest known disease and the most feared illness of humans and animals with records dating to ancient Egypt (2,300 BC), Greece (described by Aristotle), and zoroastrian Avesta in 6th century BCE Persia.⁶ Early Indian texts such as Susrutasamhita noted its transmission via rabid dog saliva, was later confirmed experimentally by Zinke in 1804.⁷ Louis Pasteur's development of the first rabies vaccine in 1885 was a major milestone and it successfully treated Joseph Meister, a boy severely attacked by an animal affected by rabies.⁸ The 20th century saw critical advancements: identification of RABV in 1903⁹, its spread among European foxes in 1940s¹⁰, and the launch of oral vaccination campaigns in 1978. By 1991, Finland was declared to be rabies free, highlighting the success of integrated control measures.¹¹

EPIDEMIOLOGY

In general, RABV occurs globally with an exception of some geographical regions such as islands. As for now, some countries including United Kingdom, Sweden, Ireland, Japan, Norway, Iceland, New Zealand, Australia, Singapore, majority of Malaysia, Papua New Guinea, most Pacific Island countries and some Indonesian islands had been free from rabies virus for many years.¹² Rabies poses a serious threat to birds, humans and their domestic animals and wildlife. Estimates of human rabies deaths globally are 30,000–

50,000 causing loss of large number of domestic animals and wildlife annually.¹³

In Europe, the red fox is a vital reservoir of RABV and increased incidence of rabies in foxes is often followed by rising cases of rabies in domestic animal like sheep, cattle, horses, dogs, and cats.¹⁴ The existence of the sylvatic (wildlife) and urban (dog-mediated) cycles substantially overlaps in some areas, while other regions have mainly the sylvatic cycles. For instance, in 2010, 90% of the animal rabies documented in the United States and Canada were because of wild animals. Rabies may cause serious conservation problems in certain species of animals that are either rare or endangered.¹⁵ In Africa, Ethiopian wolf (*Canis simensis*) and the African wild dog (*Lycaon pictus*) are endangered species that face significant threats from rabies virus.¹⁶

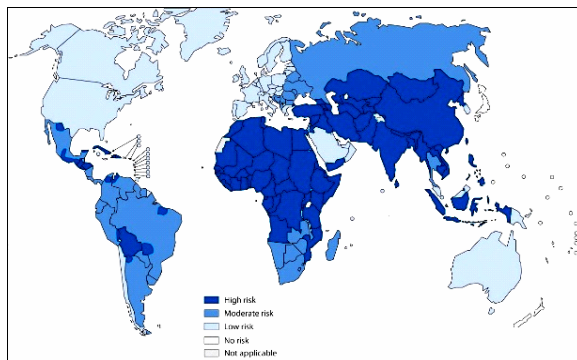


Figure-1: Geographical distribution of rabies virus¹⁷

HOST RANGE

Rabies can infect all mammals but transmission to humans and domestic animals is from certain species that serves as reservoir. These include species of the canid family (e.g., dogs, foxes, wolves, jackals, coyotes, and raccoon dogs), members of the mustelid family (e.g., skunks), members of the viverrid family (e.g., mongoose) members of the procyonid family (e.g., raccoons), and those forming the chiropteran order (bats). Rabies reservoirs are commonly divided into terrestrial species and bat species.¹⁸

Rabies is endemic in some populations and can be either accidental in man or a part of enzootic or epizootic cycles in animals. In the enzootic state, rabies persists as an endemic disease within a specific reservoir species in a particular area, maintaining a relatively stable incidence rate. An epizootic occurs when there is a sudden increase in rabies cases among the reservoir species. When rabies is inadvertently transmitted from a reservoir host species to a non-reservoir host species, the event is termed 'spill over'. Common reservoir species include the raccoon (*Procyon lotor*), gray fox, striped skunk (*Mephitis*), red fox (*Vulpes vulpes*), coyote (infected with the dog variant), and Arctic fox (*Alopex lagopus*).¹⁹

TRANSMISSION

Rabies is transmitted from the bite, scratch, or by direct contact with saliva, tears, or neural tissue of a suspected or confirmed rabid animal with an open wound or mucous membrane. While the vast majority of human rabies cases result from the bite of a rabid animal or contact of infected saliva with broken skin, rare cases have been linked to inhalation of airborne virus in laboratory settings or in bat caves densely crowded by the infected bats.²⁰

REPLICATION

The life cycle of the RABV involves a viral infection of the CNS, consists of a highly coordinated sequence of events within the host (Figure-2). It affects the host when an infected animal bites or scratches, introducing the virus through saliva into the wound. The virus initially replicates in local muscle tissues before spreading into peripheral nerve tissue and subsequently the CNS.²¹ After the virus enters the host cell it attaches itself to nicotinic acetylcholine receptors and gains entry through endocytosis.²² It releases its RNA to enter in the cytoplasm where the viral RNA dependent RNA polymerase copies the genome into a *mRNA*. This *mRNA* is translated into nucleoprotein, glycoprotein, phosphoprotein, matrix protein, and RNA polymerase which are important viral structural and replicative proteins.²³

Synthesizing new viral genomes takes place along with the production of negative sense RNA strand from which positive sense RNA genomes are synthesized. These new viral RNA and proteins go to the cytoplasm of the infected host cell, and form ribonucleoprotein complexes.²⁴ The new virions bud off from the host cell by acquiring new envelop from the host cell membrane that consists of viral glycoproteins and in the process, the host cell is often killed. Such highly ordered replication ensures the production of a large number of virions that are capable of further infecting new cells and ultimately to new hosts.¹⁷

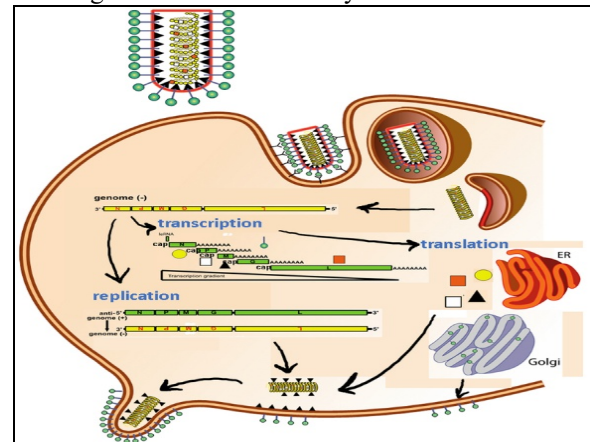


Figure-2: Replication Cycle of Rabies Virus¹⁹

PATHOGENESIS

Rabies lyssavirus typically enters the body through breaks in the skin or mucous membranes, as intact skin acts as a barrier to infection. After initial infection in muscle tissue near the entry site, the virus targets the motor neurons at neuromuscular junctions for entry into nervous system.²⁵ The virion undergo rapid retrograde axonal transport via dynein-mediated movement along microtubules. Upon reaching the CNS, the virus spreads trans-synaptically, leading to encephalitis characterized by neuronal dysfunction and apoptosis.²⁶ Fatal outcomes primarily result from brainstem involvement causing respiratory failure, often accompanied by metabolic disturbances like keto-alkalosis due to hyperventilation and neuromuscular hyperactivity.¹⁵

CLINICAL SIGNS AND SYMPTOMS

The clinical presentation of rabies in animals varies by species, individuals, and disease stage, but generally progresses through three phases.²⁷ After some time, there are clinical manifestations of the disease. The first stage commonly lasts about 1–3 days and may include minimal changes in behaviour such as aggression in normally calm animals, day time activity in animals that are active at night, the lack of fear of humans in wild animals, or abnormal eating and vocalization.²⁸ This is followed by the excitement or furious phase, characterized by increased aggression, irritability, and a tendency to bite objects, animals, or people. Affected animals may display excessive vocalization and abnormal behaviour. In some cases, seizures and hyperactivity occur, and wild animals may lose their fear of humans.²⁹ The final paralytic or ‘dumb’ phase involves progressive paralysis, starting with the hind limbs and facial muscles, leading to difficulty in swallowing, excessive drooling, and ultimately complete paralysis and death due to respiratory failure.³⁰

DIAGNOSIS

Diagnosing rabies involves a combination of clinical assessment, laboratory testing, and careful review of the patient’s exposure history. This can be challenging because early signs and non-specific and early markers are limited. Clinician assess the patient’s symptoms and medical history, with close attention to any recent animal exposure, especially bites or scratches from potentially infected animals. Early signs can include fever, headache, and discomfort or pain at site of bite. As the disease progress, more characteristic features may appear such as hydrophobia, hypersalivation, hallucinations, paralysis and seizures.³¹ The detection of rabies virus antigen or nucleic acid is both rapid and sensitive method of diagnosing rabies virus infection. Various immunoassay techniques can be used for the detection of rabies antigen which include direct fluorescent antibody (DFA), enzyme-linked

immunosorbent assay (ELISA), immunochemistry (e.g., direct rapid immunochemical test or dRIT, indirect rapid immunochemistry test or IRIT), or immunoblot (immunochromatography, dot-blot). Among them, DFA test is considered the gold standard test, which detects the presence of rabies virus antigen in infected tissues, especially in the brain.³²

PCR tests can detect rabies virus RNA in saliva, cerebrospinal liquid, or tissue specimens and is especially valuable for diagnosing rabies in living animals or humans before the appearance of clinical signs. PCR assays have high sensitivity and specificity, allowing early detection and timely intervention.³³ Serological tests, such as enzyme-linked immunosorbent test (ELISA) or rapid immuno-chromatographic tests, detect antibodies produced by the immune system in response to rabies virus exposure. These tests are valuable for screening people who are at high occupational risk of rabies, such as veterinarians, animal handlers, and laboratory personnel, and for determining whether they require a booster vaccination.³⁴ Magnetic resonance imaging (MRI) or computed tomography (CT), may be performed to evaluate neurological anomalies related with rabies diseases. These imaging procedures offer assistance visualize structural changes in the brain and spinal cord, helping in diagnosis and monitoring the disease progression.³⁵

TREATMENT

Once clinical symptoms of rabies develop, treatment is generally limited to supportive care, as there is no effective cure available at this stage. Care is focused on alleviating distress and addressing complications. Patients are often sedated to reduce pain, agitation, fever, suffering, and may require intensive supportive measures like management of paralysis, and mechanical ventilation. Ketamine, a dissociative anaesthetic, has been investigated for its potential to inhibit RABV replication in neuronal tissues and is considered a suitable agent for sedation in these cases.³⁶

Lyssaviruses are easily inactivated by exposure to sunlight, thorough washing, and aeration. Immediate and meticulous wound care is critical in rabies prevention. Studies in animal models suggest that prompt wound cleansing within the first three hours after exposure can nearly eliminate the risk of infection. The recommended procedure is to clean the affected area thoroughly for at least 15 minutes with water and antiseptic soap, followed by use of a virucidal agent such as povidone-iodine or alcohol to further reduce viral presence.³⁷ For post-exposure prophylaxis (PEP), the CDC advises administering human rabies immunoglobulin (HRIG) directly into and around the wound, using as much of the calculated dose as anatomically feasible, with any remaining volume given intramuscularly at a site distant from vaccination spot.³⁰

Table-1: Documented cases of human recovery from RABV infection

Transmission	Incubation period	Complications	Treatment	Outcome	Reference No.
Bite from a clinically rabid dog (dog died 4 days later)	21 days	Quadriparesis, cerebellum dysfunction, cardiac arrhythmia, altered consciousness	Suckling-mouse brain rabies vaccine should be administered 10 days after exposure	Recovery with two relapses following booster doses; gradual resolution over 1 year	38
Head bite by rabid dog	19 days	Encephalitis, convulsions, deep coma, quadriplegia	Vero cell-derived vaccine without rabies immunoglobulin, given the next day	Minor improvement; response to pain, persistent blindness and deafness; death after 34 months	39
Thumb bite by rabid brown bat	20 days	cardiac arrhythmia, encephalitis, coma, paralysis	Duck-embryo vaccine without rabies immunoglobulin, given the next day	Intensive care provided; complete recovery achieved in six months	40
Bites to face and hand by stray dog (dog died after 4 days)	16 days	Hallucinations, focal seizures, coma, hydrophobia	No wound cleaning and chick embryo rabies vaccine without immunoglobulin, same day	Three months in coma; slow improvement with spasticity, tremors, and involuntary movements at 18 months	41
Inhalation of aerosols containing fixed RABV in laboratory	21 days	Encephalitis, impaired consciousness, spastic hemiparesis	Pre-exposure duck-embryo cell vaccine only	Gradual improvement; long-term sequelae including personality disorder and dementia	42

PREVENTION

Rabies prevention centres on a combination of public education, animal vaccination, and prompt medical intervention following exposure. The most effective therapy is mass vaccination of dogs, as they are the main source of infection. Responsible pet ownership-including keeping pets' rabies vaccinations up to date and preventing their contact with wild or stray animals-significantly reduces risk.⁴³ Avoiding contact with unknown, sick, or wild animals, and teaching children to report animal bites, are also crucial preventive measures. If exposure occurs, immediate and thorough wound cleansing with water and soap should be done and then rabies vaccine should be

administrated and, when indicated, rabies immunoglobulin is nearly 100% effective at preventing disease onset.⁵ Community awareness campaigns and coordinated efforts between veterinary and public health authorities further strengthen rabies control and prevention.⁴⁴ Rabies is always fatal in unvaccinated humans due to neurological symptoms that appear. PEP immunization is particularly effective for disease prevention when administered promptly, ideally before the onset of symptoms. Even when there are delays or barriers to accessing PEP, initiating the treatment as soon as possible still offers a chance of preventing the development of rabies.³⁰

Table-2: Vaccine and therapeutic agents for prevention of RABV infection

Drug	Type of vaccine	Purpose	Mechanism of action	Comments	Reference No.
Inactivated rabies vaccine	Inactivated vaccine	Pre- and PEP	Induces virus-neutralizing antibodies (VNA) via B cells	Licensed in the United States; not effective as treatment after symptom onset	45
Live rabies vaccine	Live- attenuated vaccine	Oral vaccination of wildlife	Stimulate CD4, CD8 cells, innate immunity, and VNA (B cells)	Administered orally to wildlife; not approved for human rabies treatment	46
IFN-alpha	Immunoregulatory protein	Experimental treatment	Inhibits viral replication	Associated with CNS toxicity, spastic diplegia, and psychosis	47
Rabies immunoglobulin	Polyclonal VNA or monoclonal antibody cocktail	PEP	Neutralizes virus at the site of exposure	Used only for PEP; not effective as treatment for established rabies	48
Ketamine	Dissociative anaesthetic, NMDA receptor antagonist	Experimental therapy	Inhibits RABV transcription in vitro; provides neuroprotection	In vivo antiviral efficacy is inconsistent; primarily used for sedation	49

CONCLUSION AND FUTURE PERSPECTIVES

Eradication of rabies is achieved through implementation of robust diagnostic tests and early detection of disease progression. Enhanced surveillance is necessary to monitor circulating RABV variants, especially those prevalent among wildlife, as the true frequency in these populations remain unclear. Comprehensive studies are needed to elucidate the role of wild animals in transmission of rabies, along with to identify emerging or

atypical RABV variants in non-reservoir species. Given that RABV is transmissible through contact of saliva infected with rabies with broken skin or mucous membrane, strict precautionary measure are essential. It is imperative for veterinary and medical professionals to be well-informed about rabies transmission routes to effectively educate the public, particularly individuals living in endemic regions and those working in abattoirs, hence decreasing the risk of disease spread. Prompt reporting to healthcare professionals and prioritization of PEP are critical components of rabies control.

Advancements in molecular diagnostics, such as RT-PCR, have significantly improved the specificity and sensitivity of rabies detection. Early diagnostic strategies using biotechnology tools should be developed to confirm infection and differentiate between RABV genotypes. Researchers should pay attention to evolving pathology of RABV and to facilitate the development of effective antiviral therapies. Molecular biology has played a pivotal role in the production of new-generation rabies vaccines, which are crucial for controlling both human and animal cases of rabies. Children in developing countries are more vulnerable to RABV, often due to the bite of rabid dog, which contribute to endemic outbreaks. Therefore, travellers to risk areas should ensure they receive pre-exposure rabies vaccination. While oral vaccines are available, their effectiveness varies among different wildlife species, highlighting the need to assess post vaccination immune responses and vaccine stability. The development of edible vaccines for wild and domestic ruminants may further aid in rabies control. Many countries have successfully achieved rabies-free status, demonstrating that elimination is possible in high-risk areas through comprehensive preventive measures. The use of scientifically validated medicines and vaccines, combined with widespread public awareness and strong political commitment, remains essential for the global eradication of rabies.

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Received: 11 May 2025

Reviewed: 15 Feb 2026

Accepted: 25 Feb 2026

Contribution of Authors:

Authors approved the draft and are accountable in ensuring that questions related to accuracy or integrity of the work are duly investigated and resolved.

BMM: Conception and study design

AMR: Literature search

AA: Initial draft preparation

NA: Preparation of figures and tables

MH: Revision of draft and reference management

MA: Critical review, Manuscript preparation, and approval for submission

Conflict of Interest: No conflict of interest is declared,

Funding: No funds have been received from any agency



Pakistan Journal of Physiology

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