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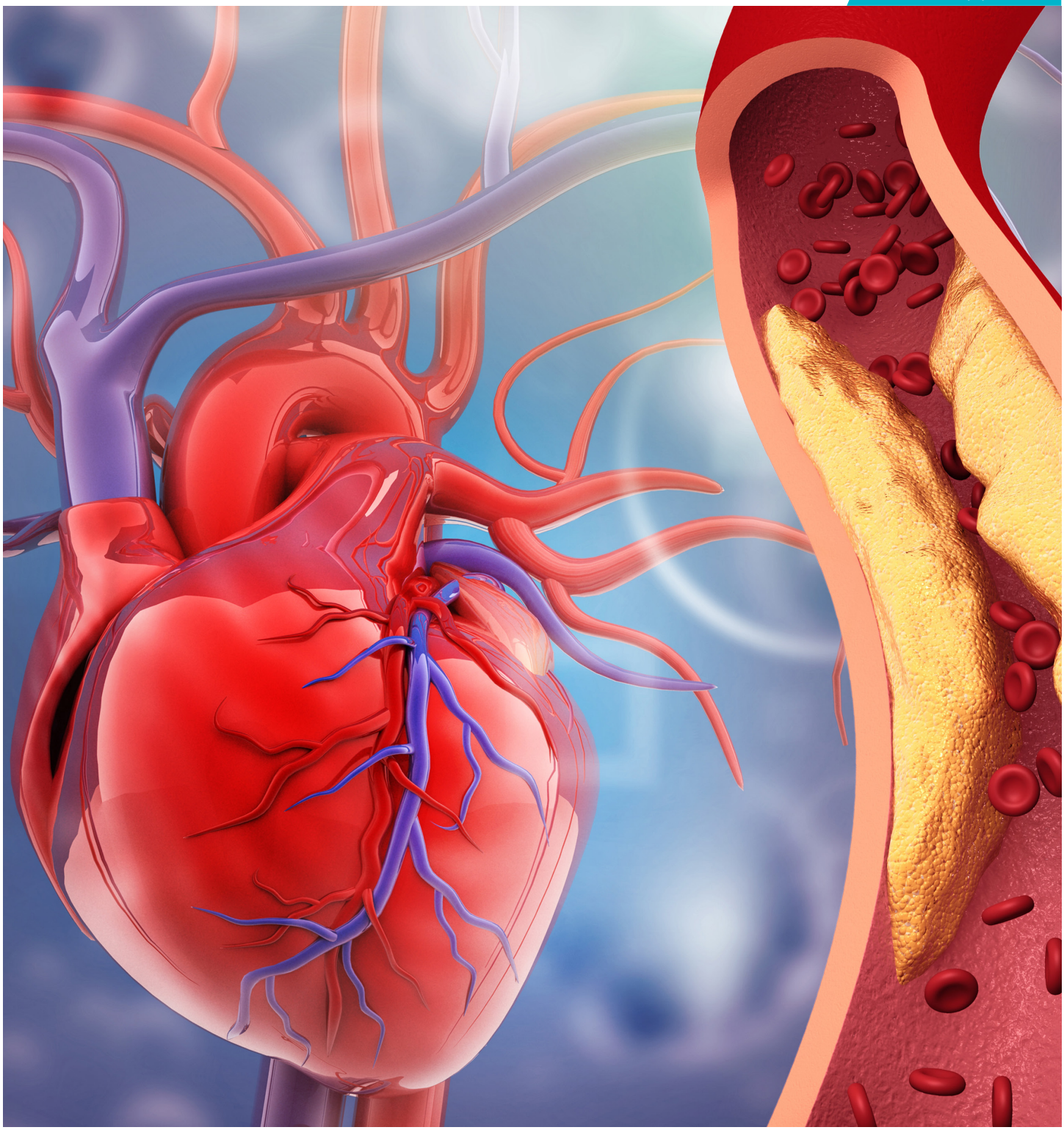

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EDITORIAL

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AIMS AND SCOPE OF THE JOURNAL

Journal "Clinical Medicine of Kazakhstan" (ISSN 1812-2892) is a multi-field dedicated peer-reviewed medical journal. The main thematic scope – publication of materials on medical science and practice, education and healthcare organization. Joint Stock Company "National Scientific Medical Center" publishes the journal bimonthly in a year (in February, April, June, August, October, and December).

All articles sent to editors undergo double-blind review. Manuscripts are judged by two experts exclusively on the basis of their contribution to initial data, ideas and their presentations. Editors accept articles for consideration and publication at no cost. Detailed information is available in the section Information for authors at the end of this material.

The Journal of "Clinical Medicine of Kazakhstan" to the full extent is wedded to initiative of open access and ready to provide free access to full texts of articles, as soon as they will be published and available in the Internet (www.clinmedkaz.org).

Journal was registered in the Ministry of Information of the RK on 05.04.2004 and currently included to the list of Publications, approved by the Committee for Control of Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan for publication of the main outcomes of scientific activity.

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Acknowledgment to JCMK Editorial Board and Peer-Reviewers for contribution in 2025

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Abstract

The publication summarizes the results of the activities for the Journal of Clinical Medicine of Kazakhstan in 2025. It was prepared on behalf of the editorial team of the Journal to express appreciation to all editorial and advisory board members, reviewers and authors who contributed to this journal throuout the year.

The year 2025 marked a significant milestone for our journal—the start of indexing in Scopus. This opens new opportunities to enhance the scientific level and international recognition of this journal, which has become an important platform for publishing clinical research results and medical advances in Kazakhstan and beyond.

On behalf of the editorial team of the Journal of Clinical Medicine of Kazakhstan, we would like to express our appreciation to all editorial and advisory board members, reviewers and authors who contributed to this journal in year 2025.

We wish all authors and the journal's team success in their work, new discoveries and achievements.

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OUR STATISTICS

2025 IN NUMBERS

Journal of Clinical Medicine of Kazakhstan published 6 regular issues in 2025:
– Volume 22, Number 1 (2025) with 13 articles;
– Volume 22, Number 2 (2025) with 11 articles;
– Volume 22, Number 3 (2025) with 14 articles;
– Volume 22, Number 4 (2025) with 14 articles;
– Volume 22, Number 5 (2025) with 16 articles;
– Volume 22, Number 6 (2025) with 15 articles.
The acceptance rate of articles in 2025 was 20%: 80 articles received in 2025 were accepted for publication and 320 articles were rejected. The remaining 3 articles published in 2025 were received in previous year and were taken into account in the statistics for the relevant period.

AUTHORS 2025

Authors and coauthors who contributed to this journal in 2025 were from the following countries: Azerbaijan, Brazil, India, Indonesia, Iraq, Japan, Kazakhstan, Nigeria, North Macedonia, Turkey, USA.

The editorial team of the Journal of Clinical Medicine of Kazakhstan would like to express gratitude for your valuable support and being part of our excellent team. We appreciate your continuous efforts and hope to continue receiving your great feedback, valuable ideas, and interesting scientific papers to further improve the quality and impact of the Journal of Clinical Medicine of Kazakhstan.

In addition, we would like to express heartfelt thanks to all those who contributed to the editorial process and the successful indexing of the journal in Scopus.

Sincerely yours,
Editorial team of the Journal
of Clinical Medicine of Kazakhstan

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Incidence and Risk Factors of Retinopathy of Prematurity (ROP) – A Prospective Study in a Tertiary Care Centre-Patna

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Abstract

Aim: Retinopathy Of Prematurity is a complex disease of developing retinal vasculature that primarily affects one or both eyes of premature babies. There are certain risk factors for ROP like RDS, CPAP, Mechanical Ventilation, Prolonged oxygen supplementation and few others. Screening of high-risk neonates at the right time will lead to early diagnosis and treatment thus preventing blindness due to ROP.

Methods: After getting approval from ethical committee, this study was carried out from 1-9-2019 to 31-8-2020. It included 177 neonates based on inclusion criteria. Babies were screened by ophthalmologist trained in ROP screening. Pupils were dilated using 0.5% Tropicamide+2.5% Phenylephrine eye drops till dilation was obtained. To study the incidence of ROP and associated risk factors of ROP in, (a)Preterm with gestational age of ≤ 34 weeks and/or birth weight < 1750 gm. (b) Selected preterm with birth weight 1751-2000gm and/or 34-36 weeks who are at high risk due to associated risk factors.

Results: The present study observed the incidence of ROP as 29.94%. On univariate analysis, Prematurity (P<0.001), Low Birth Weight (P<0.001), Supplemental Oxygen (P<0.001), respiratory distress syndrome (RDS), Apnea of Prematurity (AOP) (P<0.001), Blood transfusion (P<0.001), Sepsis (P<0.001), Feed Intolerance (P<0.001), Phototherapy (P<0.001), and Singleton Pregnancy (P<0.01) have risk factors for ROP.

Conclusion: Reducing the incidence of ROP may be achievable through the prevention of prematurity, careful management of at-risk infants, and minimization of contributing risk factors. Effective screening and timely intervention will prevent the progression of ROP to end stages thus decreasing the incidence of Blindness.

Keywords: Retinopathy of prematurity, blindness, premature babies, pupils.

Introduction

Retinopathy of prematurity (ROP), formerly known as retrolental fibroplasia, is a multifactorial condition involving abnormal development of the retinal blood vessels, typically impacting both eyes of premature infants [1-3]. In normal babies, retinal vessel extends up to the complete periphery of the total retina one month after birth. In premature baby's nasal retina gets vascularised by about 32 weeks and temporal retina by about 37-40 weeks of gestation. Damage to the developing retinal blood vessels can disrupt their normal growth, triggering the release of vascular growth factors that promote abnormal new vessel formation. This process may either result in successful revascularization and spontaneous regression of ROP or lead to the formation of neovascular membranes in the vitreous, which can cause scarring and ultimately retinal detachment [3].

The risk factors associated with ROP are not fully known. It is consistently linked to factors such as low

birth weight, reduced gestational age, and extended exposure to supplemental oxygen, septicaemia, apnea, use of blood products, longer duration of NICU stays, hyper- bilirubinaemia, hypocalcaemia, high ambient light, Vitamin E deficiency, shock, hypoxia, acidosis, anaemia, Patent Ductus Arteriosus (PDA) and extended use of ventilatory support—particularly when associated with periods of hyperoxia and low carbon dioxide levels (hypocapnia)—have also been implicated as contributing factors. Breastfeeding is protective against development of ROP [4-7]. Newborns of mothers with pregnancy-induced hypertension may have a lower risk of developing ROP, possibly due to the accelerated maturation of fetal retinal blood vessels [7].

ROP is a significant yet preventable cause of childhood blindness. Its occurrence can be lowered through timely screening and appropriate referral for treatment. Recent studies from India have indicated that the incidence of ROP varies between 20% and 30% [9-11]. International studies demonstrate marked global variation in the incidence of retinopathy of prematurity (ROP), largely influenced by gestational age, birth weight, quality of neonatal care, and associated clinical risk factors. In the United States, the CRYO-ROP Group Study reported ROP in 66% of infants weighing less than 1,250 g and in 82% of those weighing less than 1,000 g [12].

In Iran, Azami et al. reported an overall ROP incidence of 23.5% in 2018 [13,14]. From Africa, the prevalence of vision-threatening ROP was reported to be 20.9% in Kenya [15,16], while in Nigeria a 2015 study reported a prevalence of 15%, with half of the affected infants requiring treatment [16,17]. In Sweden, ROP was observed in 18% of infants with birth weight <1,500 g and 24% of those <1,000 g, with incidence rising to 61% in infants born before 27 weeks of gestation [18,19]. Similarly in other European and developed nations, ROP was reported in 33% of infants born before 28 weeks in Norway, severe ROP in 26% in Belgium and 16% in Austria of infants <27 weeks, and 5%–10% in infants weighing <1,000 g in Finland; in Australia and New Zealand, severe ROP was identified in 10% of infants born before 29 weeks of gestation [19].

In Asia and Latin America, reported prevalence ranges widely, including 14%–33% in the Philippines, 15.5% in Venezuela, 40%–50% in Romania, and 40.7% in Thailand [20]. In South Africa, ROP prevalence was reported as 19.2%, with a notable reduction in treatable ROP following implementation of ROP screening protocols, while Sudan reported a prevalence of 37% [16]. In Indonesia, ROP prevalence ranged from 11.9% to 30.5% among infants born <32 weeks and weighing <1,500 g [21], while lower incidence rates were reported in Ghana (13.7%) and higher rates in Saudi Arabia (33.3%) [22,23].

Additional international studies further reinforce these trends, with Chyne (1999) reporting a 15% incidence among infants ≤37 weeks and ≤1,250 g [24], Fledelius (2000) reporting 10% among infants ≤32 weeks and ≤1,500 g [25], and Bayat-Mokhtari (2010) reporting 32.6% in infants <1,500 g and in heavier infants with unstable clinical conditions [26]. Rania (2017) observed a notably high incidence of 59% in infants born before 37 weeks [27], while Ahmet (2018) and Freilas (2018) reported incidences of 27% and 33.9%, respectively, particularly among very preterm and low-birth-weight infants, with additional risk factors such as respiratory distress, blood transfusion, intraventricular hemorrhage, multiple gestation, and sepsis contributing to disease occurrence even in more mature infants [28,29]. Khorshidifar (2019) reported an incidence of 27% among infants born before 34 weeks with birth weight <2,000 g [30]. Collectively, these findings highlight that although prematurity and low birth weight are the strongest predictors of ROP, clinically unstable infants with higher gestational age and birth weight also remain at significant risk, underscoring the importance of inclusive and comprehensive screening strategies worldwide. Because ROP can advance rapidly and delay in treatment adversely affects chances of success, ROP screening guidelines, early detection and timely treatment along with improved maternal and neonatal care can control the epidemic [31-33].

At present, choice of treatment for ROP is laser photoablation of non-vascularised immature retina [34]. Other modalities of treatment are intra-vitreal injection of Bevacizumab (anti-VEGF drug), parsplana vitrectomy, circlage and supplementation of vitamin E as prophylaxis [35-38]. This prospective study, tried to find out the incidence and risk factors for ROP in preterm admitted in Kurji Holy Family Hospital (KHFH) over a period of 1 year. ROP is a preventable and treatable cause of blindness in infant and not much study has been done in Bihar.

Methods

1. Ethical approval: Ethical clearance was granted with IEC Letter No. RMRI/EC/33/2019 dated August 30, 2019, by the Institutional Ethics Committee of the Indian Council of Medical Research Unit – Rajendra Memorial Research Institute of Medical Sciences (MoU for Ethics approval), Patna, Bihar, India. This approval applies to all aspects of the research activity. The objectives of the research were explained to all patients, and their written informed consent was acquired before the inquiry started. All procedures used in the study were compliant with the ethical norms outlined by the Indian Council of Medical Research and the Government of India regarding the use of human subjects in research.

2. Study area: The study was conducted in the Department of Paediatrics,

Kurji Holy Family Hospital, a tertiary care referral hospital for children.

3. Study design: Prospective Observational Cohort Study.

4. Study population: Study group comprised of premature and low birth weight babies admitted in the NICU (Neonatal Intensive Care Unit) and general neonatal ward of hospital (according to our inclusion criteria).

5. Inclusion criteria:

(a) Babies admitted with Gestational age ≤ 34 weeks

(b) Selected preterm infant between 34-36 weeks with birth weight 1751-2000gm who were at a higher risk of developing ROP like those who had

- Prolonged oxygen therapy
- Respiratory distress syndrome
- Assisted Ventilation
- Apnea of prematurity,
- Anemia needing blood transfusion
- Neonatal sepsis
- Intraventricular hemorrhage

6. Exclusion criteria:

- Infants suspected of having chromosomal abnormalities
- Infants who passed away before complete development of the retinal blood vessels
- Babies with major congenital malformation

7. Sample size

The study of Chaudhari et al. (2009) [5] observed that incidence of ROP was 22.3%. Taking this value as reference, the minimum required sample size with 6.5% margin of error and 5% level of significance is 158 patients. Also, oxygen therapy, apnea, were the significant risk factors of ROP with odds ratio of 2.75, 5.19. Taking these values as reference, the minimum required the calculated sample size for the study, based on

80% statistical power and a 5% level of significance, was 163 participants. However, the current study included a total of 177 patients.

Formula used is:

$$n \geq ((i\ (1 - i))/(ME/z_{\alpha})^2 \quad (1),$$

where Z_{α} is value of Z at two-sided alpha error of 5%, ME is margin of error and i is incidence rate.

$$n >= (4*(Z_{\alpha}+Z_{\beta})^2)/ (\log (OR))^2 \quad (2),$$

where Z_{α} is value of Z at two-sided alpha error of 5% and Z_{β} is value of Z at power of 80% and OR is Odds Ratio.

8. Methodology:

After getting approval from the institution's ethical committee and on the basis of inclusion criteria, this study included 177 newborns as sample size. The written informed consent was obtained from parents/ legal representative of children after explaining the study procedure to their satisfaction. A standard care record form was maintained for each subject.

The screening was done with a binocular indirect ophthalmoscope. Topical anesthesia used was 2% Proparacaine drops.

- The initial screening was conducted within 4 weeks after birth, or by the 30th day of life, for infants with a gestational age of 28 weeks or more.
- Infants with a gestational age below 28 weeks or a birth weight under 1200 grams were screened earlier, at 3 weeks of age, to facilitate the prompt detection of aggressive posterior ROP [3].

Pupil dilation was achieved by administering a combination of 0.5% Tropicamide and 2.5% Phenylephrine eye drops two to three times, until complete dilation was attained. Special attention was given to the possibility of bradycardia, arrhythmia, asystole, hypoventilation, apnea, or aspiration. Hence, every infant was observed during the examination with a NICU staff member in attendance. Retinopathy was classified by stage and zone according to the ICROP guidelines. Follow-up evaluations were conducted as advised by the examining ophthalmologist. Following is the IAP suggested schedule for follow-up intervals for the infants at risk [3].

Table 1 IAP Suggested schedule for follow-up intervals

when to follow-up repeat ROP examination	
retinal finding	**follow-up schedule**
Stage 1 or 2 ROP: Zone I Stage 3 ROP: Zone II	1 Week or less
Immature vascularization: Zone I - no ROP Stage 2 ROP: Zone II Regressing ROP:Zone I	1-2 Weekly
Stage 1 ROP: Zone II Regressing ROP:Zone II	2 Weekly
Immature vascularization: Zone II - no ROP Stage 1 or 2 ROP: Zone III Regressing ROP:Zone III	2-3 Weekly

All at-risk infants continued to undergo regular ROP screening until one of the following occurred [39]:

a. The retina was fully vascularized.

b. ROP had completely resolved with no remaining signs indicating a risk of vision loss, which typically occurred around 40 to 42 weeks of postmenstrual age.

c. Postmenstrual age of 45 weeks and no pre-threshold disease.

d. ROP has progressed to a level of severity where treatment was indicated.

e. Zone III retinal vascularization without previous zone I or II ROP.

Treatment of ROP was done by our Consultant Ophthalmologist as per ETROP guidelines.

9. Study period: 1year from 1st September 2019 to 31st August 2020.

10. Statistical analysis:

Categorical variables are expressed as counts and percentages (%), while continuous variables are reported as mean ± standard deviation (SD) and median. The Kolmogorov-Smirnov test was used to assess the normality of the data. If the data did not follow a normal distribution, non-parametric tests were applied. Statistical analyses were carried out as follows:

1. Quantitative variables were compared between ROP-positive and ROP-negative groups using the Unpaired t-test for normally distributed data, or the Mann-Whitney test when the data did not follow a normal distribution.

2. Qualitative variables were analyzed using the Chi-Square test or Fisher's Exact test, depending on the data distribution and sample size.

3. Univariate and multivariate logistic regression analyses were performed to identify the significant risk factors associated with ROP.

A p-value less than 0.05 was considered indicative of statistical significance.

Data entry was carried out using an MS Excel spreadsheet, and statistical analysis was performed using SPSS software, version 21.0 (Statistical Package for the Social Sciences).

Results

In the current study, 177 neonates meeting the inclusion criteria for ROP were screened over a one-year period. Among them, 53 were diagnosed with Retinopathy of Prematurity, resulting in an incidence rate of 29.94%. (Figure 1A). Out of 98 males, 34 (27.2%) developed ROP and among 79 females 19 (36.5%) developed ROP. The association between Sex and ROP is considered to be statistically insignificant (p-value-0.124) [Value – 2.362, D.F – 1, p – Value - 0.124] (Figure 1B). The incidence of ROP was 100% in babies <28 weeks of gestational age, 93.7% between 28-30 week of gestational age and 46.5% in the GA group 30-32 week. A lower gestational age was identified as a significant contributing factor to the development of ROP ($p<0.001$) [Value –62.56, D.F – 4, p – Value < 0.001] (Figure 1C). A strong correlation was found between lower birth weight and a higher incidence of ROP ($p < 0.001$). Among infants classified as Extremely Low Birth Weight (≤ 1000 grams), the incidence of ROP was 100%. In comparison, 62.9% of neonates in the Very Low Birth Weight category (1001–1500 grams) developed ROP, with 34 out of 54 affected. \[Chi-square value: 71.054, degrees of freedom: 7, $p < 0.001$] (Figure 1D). Out of 14 babies who received Mechanical Ventilation, 11 babies (78.5%) developed ROP. Mechanical Ventilation was a significant risk factor for the development of ROP ($p <0.001$) [Value – 17.137, D.F – 1, p – Value < 0.001] (Figure 1E). Among the 59 infants who were administered CPAP, 42 (71.1%) developed ROP. The use of CPAP was found to be a significant risk factor for the onset of ROP ($p < 0.001$) [Chi-square value: 71.76, degrees of freedom: 1, $p < 0.001$]. (Figure 1F).

Out of 63 babies who received Nasal Prongs, 38 babies (60.32%) developed ROP. Nasal Prongs was a significant risk

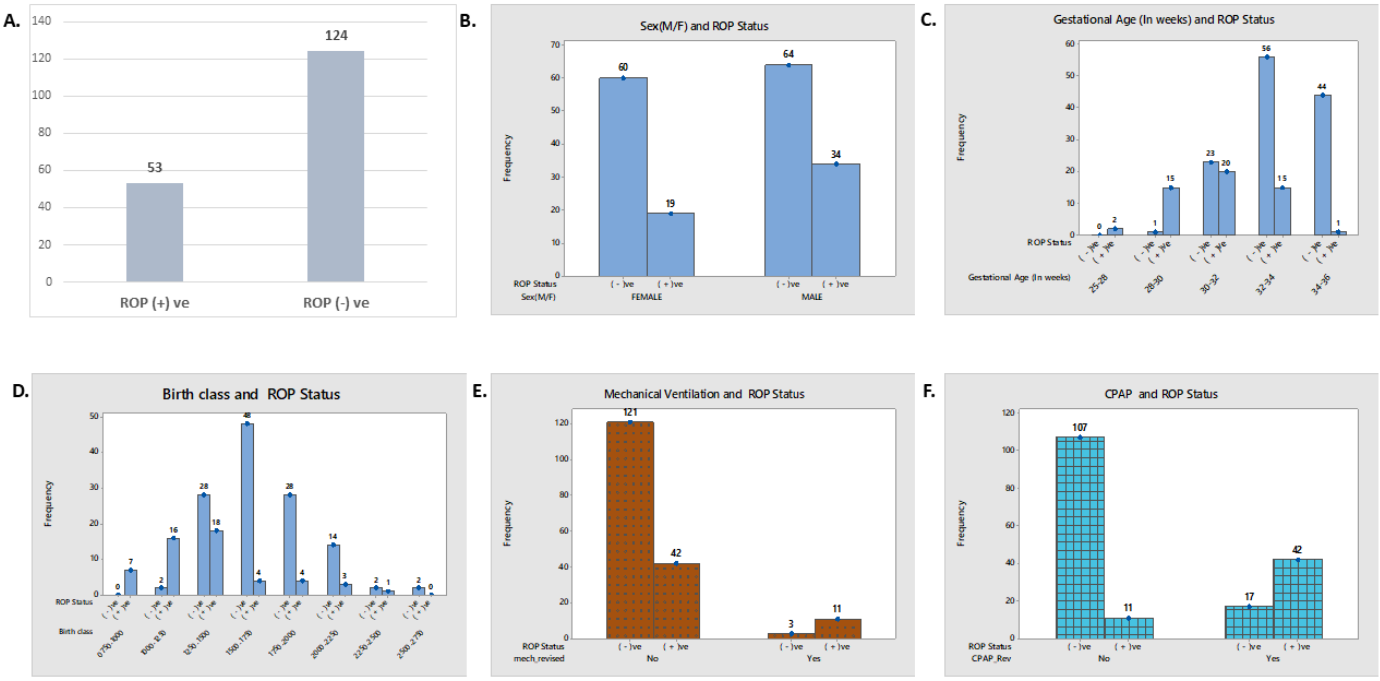

Figure 1 – 1A: Incidence of ROP; 1B: Distribution of sex and ROP; 1C: Distribution of gestational age (in weeks) and ROP; 1D: Distribution of birth weight (in grams) and ROP; 1E: Distribution of mechanical ventilation and ROP; 1F: Distribution of CPAP and ROP

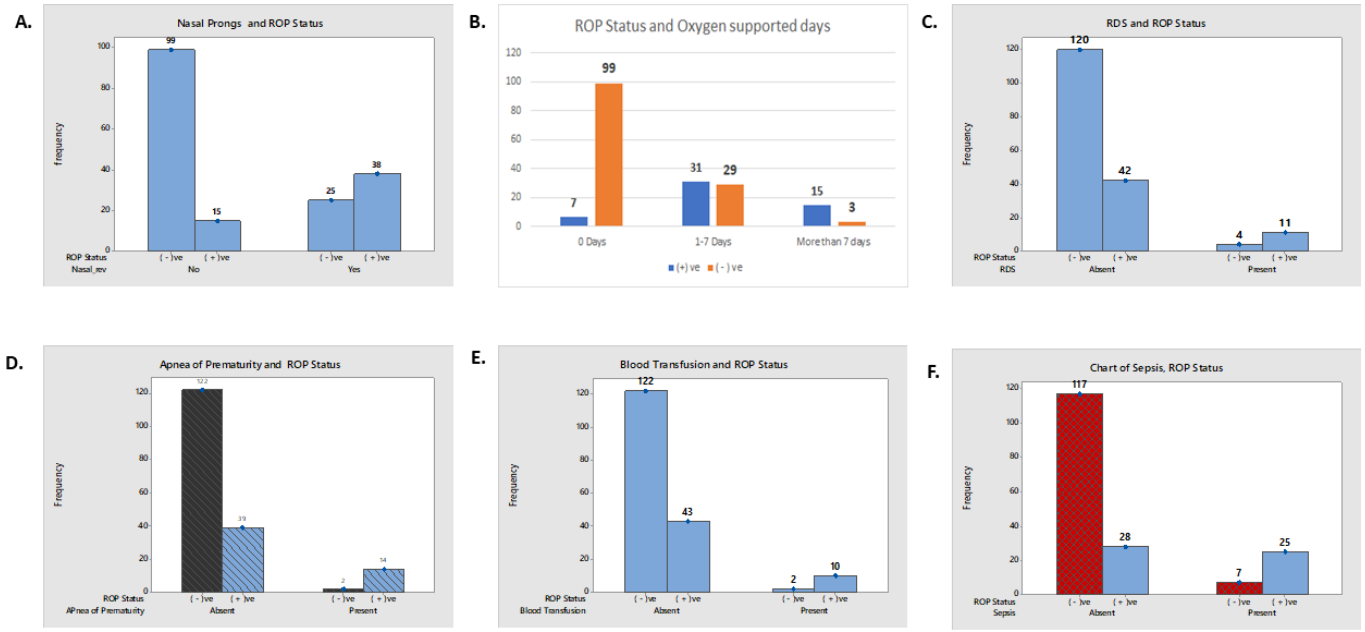

Figure 2 – 2A: Distribution of nasal prongs and ROP; 2B: Distribution of number of oxygens supported days and ROP; 2C: Distribution of RDS and ROP; 2D: Distribution of apnea of prematurity and ROP; 2E: Distribution of blood transfusion and ROP; 2F: Distribution of sepsis and ROP

factor for the development of ROP (p <0.001) [Value – 43.019, D.F – 1, p – Value < 0.001] (Figure 2A). Out of 18 neonates who received oxygen for more than 7 days, 15 (83.3%) developed ROP. Similarly, out of 60 neonates who received oxygen in the range of 1-7 days, 51.6% developed ROP whereas out of 99 babies in room air, only 7 neonates (7%) developed ROP. Thus, more the oxygen supported days, more the likelihood of developing ROP. Oxygen was a significant risk factor for the development of ROP (p <0.001) [Value – 62.64, D.F – 2, p – Value < 0.001] (Figure 2B). Out of 15 babies who had RDS and received surfactant, 11 babies (73.3%) developed ROP. RDS requiring surfactant therapy was a significant risk factor for the development of ROP (p –value<0.001) [Value – 14.709, D.F – 1, p – Value < 0.001] (Figure 2C). Out of 16 babies who had Apnea of Prematurity and required treatment for it, 14 babies (87.5%) developed ROP. Apnea of prematurity was significantly associated with the development of ROP (p < 0.001). Statistical analysis showed a Chi-square value of 27.78 with 1 degree of freedom, and Fisher's exact test also confirmed the significance with a p-value < 0.001 (Figure 2D). Out of 12 babies who received any one of the Blood products, 10 babies (83.3.%) developed ROP. Blood Transfusion was a significant

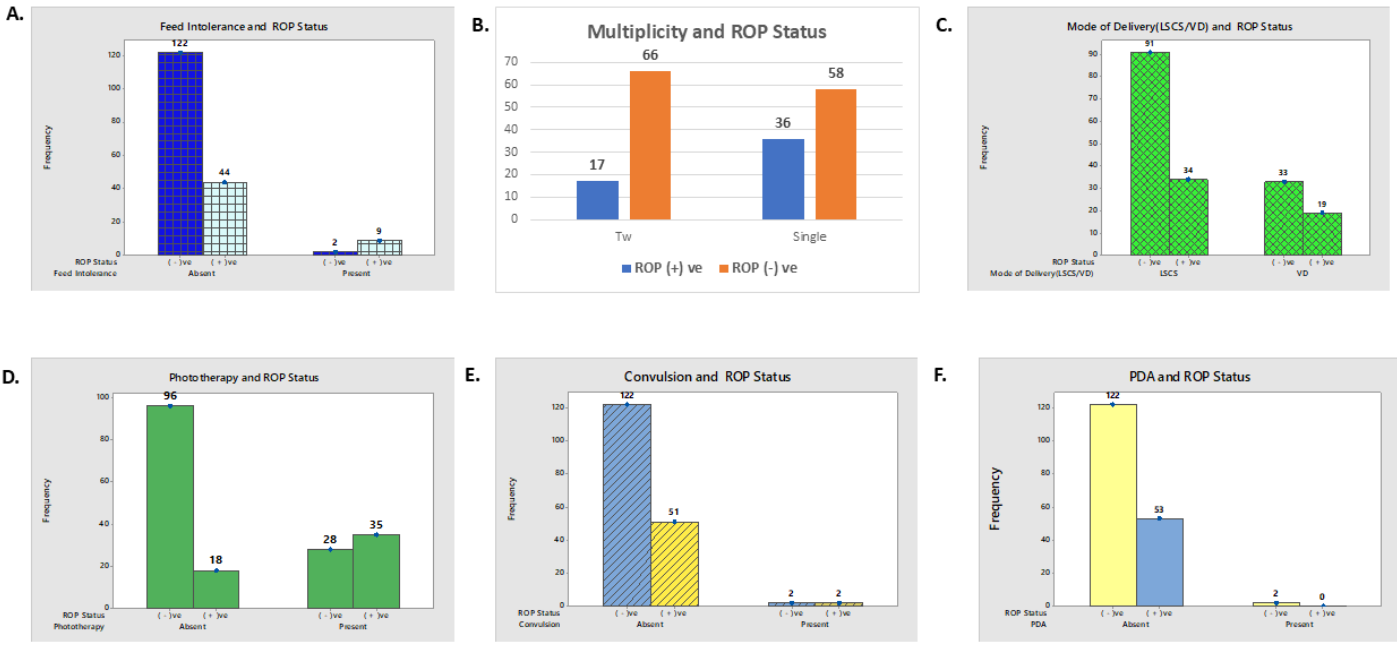

Figure 3 – 3A: Distribution of feed intolerance and ROP; 3B: Distribution of twin/singleton pregnancy and ROP; 3C: Distribution of mode of delivery and ROP; 3D: Distribution of phototherapy and ROP; 3E: Distribution of convulsion and ROP; 3F: Distribution of PDA and ROP

risk factor for the development of ROP (p <0.001) [Value – 17.492, D.F – 1, p – Value -<0.001], Fisher's exact test p -value <0.001 (Figure 2E). Out of 32 babies who had sepsis, 25 babies (78.1%) developed ROP. Sepsis was a significant risk factor for the development of ROP (p <0.001) [Value – 43.29, D.F – 1, p – Value < 0.001] (Figure 2F).

Out of 11 babies who had Feed Intolerance, 9 babies (81.8.%) developed ROP. Feed Intolerance was a significant risk factor for the development of ROP (p <0.001) [Value – 15.046, D.F – 1, p – Value < 0.001], Fisher's exact test p - value <0.001(Figure 3A). Out of 83 twin babies, 17 babies (20.48%) developed ROP whereas out of 94 single babies, 36 babies (38.3%) developed ROP. In our study, Singleton Pregnancy was a significant risk factor for the development of ROP (p value-0.01) [Value – 6.67, D.F – 1, p – Value - 0.01] (Figure 3B). Out of 125 LSCS deliveries, 34 babies (27.2%) developed ROP whereas out of 52 Vaginal deliveries 19 babies (36.5%) developed ROP. The association between mode of delivery and ROP is considered to be statistically insignificant (p-value -0.217) [Value – 1.527, D.F – 1, p – Value - 0.217] (Figure 3C). Out of 63 babies who had received phototherapy, 35 babies (55.5%) developed ROP. Phototherapy was a significant risk factor for the development of ROP (p –value<0.001) [Value – 30.588, D.F – 1, p – Value < 0.001] (Figure 3D). Out of 4 babies who had convulsion, 2 babies (50%) developed ROP. The association between Convulsion and ROP is considered to be statistically insignificant (p-value -0.376) [Value – 0.785, D.F – 1, p – Value - 0.376], Fisher's exact test p-value = 0.584 (Figure 3E).

Out of 2 babies who had significant PDA requiring treatment with Paracetamol, none of the babies developed ROP. The association between PDA and ROP is considered to be statistically insignificant (p-value -0.352) [Value – 0.865, D.F – 1, p – Value - 0.352], Fisher's exact test: P-Value = 1 (Figure 3F). Out of 2 babies who had Intraventricular Hemorrhage, 1 baby (50%) developed ROP. The association between Intraventricular Hemorrhage and ROP is considered to be statistically insignificant (p-value -0.491) [Value – 0.475, D.F – 1, p – Value – 0.491], Fisher's exact test: p-Value = 0.51 (Figure 4A). Out of total 53 ROP cases, 37 cases required Laser while 16 cases were self-regressed (Figure 4B).

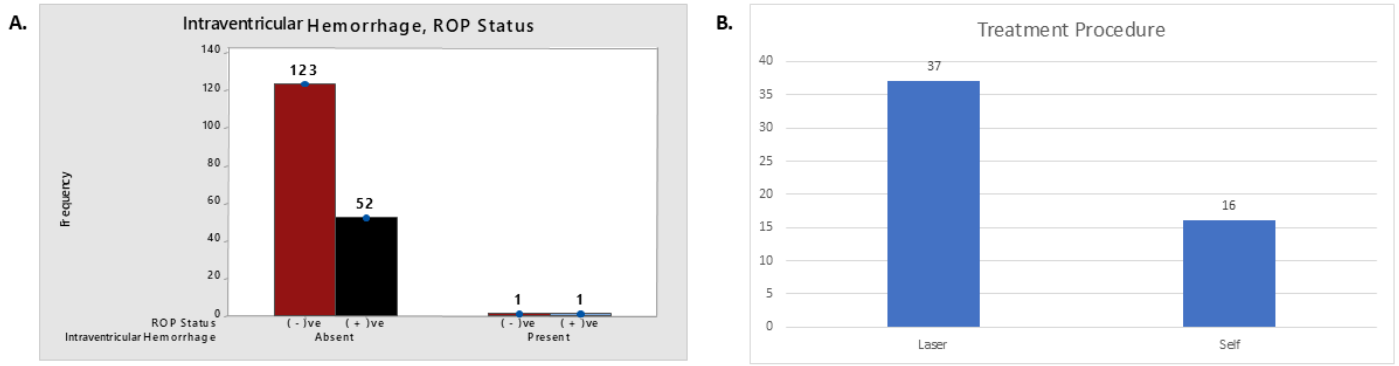

Figure 4 – Distribution of intraventricular haemorrhage and ROP; 4B: Distribution of laser and self-regressed ROP

Table 2 Multivariate logistic regression of risk factors of ROP

Factor	OR (95 % CI)	P-value
Gestational Age (<34 weeks)	1.0217 (0.012-5.475)	<0.001
Birth Weight (<1250 gm)	1.26 (0.043-22.12)	<0.001
CPAP	29.08 (10.41-55.23)	<0.001
Blood Transfusion	15.149 (1.13- 57.34)	0.017
RDS	8.53 (2.87-17.54)	<0.001

Table 3 Univariate Logistic Regression for ROP

Risk Factor	Odds Ratio	Confidence Interval	P-Value
Gestational Age			
<28	2.834	1.980-4.45	0.0295
28-31	1.245	1.034-3.432	<0.0001
31-34	1.0036	0.011-1.684	0.0202
>=34	Reference		
Birth Weight			
2250-2750	0.0217	0.0016-0.3001	0.0043
1750-2250	0.0145	0.0028-0.0756	<0.0001
1250-1750	0.0252	0.0055-0.1152	<0.0001
750-1250	Reference		
Mechanical Ventilation			
Yes	10.56	2.81-39.7	<0.001
No	Reference		
CPAP			
Yes	24.03	10.39-55.56	<0.001
No	Reference		
Nasal Prongs			
Yes	10.032	4.78-21.05	<0.001
No	Reference		
RDS			
Yes	7.857	2.37-26.01	<0.001
No	Reference		
Apnea of Prematurity			
Yes	21.8974	4.76-100.604	<0.001
No	Reference		
Blood Transfusion			
Yes	14.186	2.98-67.34	<0.001
No	Reference		
Sepsis			
Yes	14.92	5.86-37.98	<0.001
No	Reference		
Feed Intolerance			
Yes	12.47	2.59-60.00	<0.001
No	Reference		
Multiplicity			
Twins	0.4078	0.21-0.80	0.008
Single	Reference		
Phototherapy			
Yes	6.67	3.286-13.524	<0.001
No	Reference		

On multivariate analysis (Stepwise Logistic Regression) of risk factors for ROP were GA <34 weeks (P<0.001), Birth Weight <1250 gm (P<0.001), CPAP (P<0.001), Blood Transfusion (P<0.017) and RDS (P<0.093) (Table 2).

On univariate analysis, Prematurity (P<0001), Low Birth Weight (P<0.001), Supplemental Oxygen (P<001), respiratory distress syndrome (RDS), Apnea of Prematurity (AOP) (P<0.001), transfusion (P<0.001), Sepsis (P<0.001), Feed Intolerance (P<0.001), Phototherapy (P<0.001), Blood and Singleton Pregnancy (P<.01) have risk factors for ROP (Table 3).

Discussion

The importance of ROP screening stems from the fact that it is the leading cause of vision loss in children and has the potential to cause permanent visual impairment or blindness if not detected and managed early [40]. As neonatal units become more advanced, equipped with cutting-edge technology and skilled healthcare professionals delivering high-quality care to extremely premature infants, the incidence of ROP has been increasing largely due to the improved survival rates of these vulnerable newborns [41]. Primary prevention of ROP involves reducing exposure to antenatal, perinatal, and postnatal risk factors that are believed to contribute to its increased incidence and severity. Secondary prevention focuses on timely screening and prompt treatment to avoid blindness in severe ROP cases that might otherwise go undetected. As a result, the WHO VISION 2020 initiative places strong emphasis on secondary prevention strategies for ROP [8].

Incidence of ROP varies in different neonatal units. The incidence of ROP in the present study was 29.9% which is comparable to the study done by Chaudhari et al. (2009) who found an incidence of 22.3% [5]; Nikhil et al. (2016) found ROP incidence of 19.2% [41] and Patel et.al (2019) found incidence of 24.1% in their study [42]. A few western studies have also shown comparable results. The study done by Bayat-Mokhtari et al. (2010) found an incidence of 32.6% [26] Freilas et al. (2018) found ROP incidence of 33.9% [28] and Khorshidifar et al. (2019) found incidence 27% [30]. Among the 177 neonates screened, 98 (55.36%) were male and 79 (44.64%) were female. Of the 53 infants diagnosed with ROP, 34 (64.15%) were males and 19 (35.85%) were females. No statistically significant association was observed (p-value = 0.124). In a study conducted by Patel et al. (2019), among 286 neonates, 161 (56.3%) were male and 125 (43.7%) were female. Of the male infants, 38 (23.6%) developed ROP, while 31 (24.8%) of the female infants were affected. The study also reported no significant difference in ROP occurrence between male and female neonates (p-value = 0.814) [42] which is similar to our study.

A significant statistical association was found between ROP incidence and prematurity, as indicated by the chi-square test (p-value = 0.001). Interestingly, out of 2 neonates who fell in the 25-28 weeks GA group, 100% had ROP and out of 16 neonates who fell in the 28-30 weeks GA groups, 15 (93.7%) had ROP. While 20 out of the 43 neonates (46.5%) had ROP in the 30-32 weeks GA groups and 15 out of 71 neonates (21.1%) had ROP in the 32-34 GA groups. Thus, prematurity is a single most important risk factor for ROP. In a comparable study conducted by Nikhil et al. (2016), the incidence of ROP was reported at 83.3% in infants born before 28 weeks of gestation. For those born between 28 and 32 weeks, the incidence dropped to 8.3%. The study identified lower gestational age as a significant risk factor for the development of ROP (p < 0.001) [41]. Patel et al. (2019) found the incidence of ROP as 43.1% (47 out of 109) in GA ≤34 weeks and in GA >34 weeks ROP was 12.4% (22out of 177). The variation in ROP incidence between neonates with a gestational age of ≤34 weeks and those >34 weeks was found

to be statistically significant at a 95% confidence level (P value <0.001, : 34.709) [42].

Birth weight is recognized as a key risk factor for ROP, with lower birth weights associated with a higher likelihood of developing the condition. In our study, a statistically significant correlation between birth weight and ROP was established through logistic regression analysis. The incidence of ROP across different birth weight categories was as follows: 100% in the 751–1000 gm group, 88.9% in the 1001–1250 gm group, 36% in the 1251–1500 gm group, 6.9% in the 1501–1750 gm group, 12.1% in the 1751–2000 gm group, 17.6% in the 2001–2250 gm group, and 33.3% in the 2251–2500 gm group. This indicates a highly significant association between birth weight and the development of ROP.

Birth weight usually correlates with maturity of the newborn. ROP positive cases in birth weight ≤1500 grams were 41 out of 71 babies (57.74%) and in birth weight >1500 grams, 12 out of 106 babies (11.32%). The study result is similar to the study done by Patel et al. (2019) in which ROP positive cases in birth weight ≤1500 grams were 35out of 54 cases (64.8%) and in birth weight >1500 grams 34 out of 232 cases (14.7%). It was also statistically significant at 95% confidence interval (P value <0.001, : 60.207) [42].

In our study, there was a slight increase in the incidence of ROP in birth weight 1751-2000 gm, 2000-2250 gm, and 2250-2500 gm. All these cases were on either CPAP or SIMV or Prolonged oxygen therapy thus increasing their risk factors for ROP. According to AAP guidelines, ROP screening should be done in babies with GA ≤32 weeks and Birth weight ≤1500 gm. Had we followed this, we would have missed 12 cases of ROP.

In our study there is a high significant correlation between O2 supplementation and ROP (p-value<0.001). Of the neonates who required oxygen support in the form of Mechanical Ventilation, CPAP and Nasal Prongs ,78.5%, 71.1% and 60.3% respectively had ROP. Similarly, when duration of oxygen support was considered in any form of oxygen supplementation, of those neonates who required oxygen support for more than 7 days, ROP developed in 83.3% cases and those on oxygen supplementation for 1-7days developed ROP in 51.6% cases whereas only 7% of those not exposed to O2 developed ROP. In the study conducted by Nikhil et al. (2016), oxygen supplementation was identified as a significant risk factor for the development of ROP ($p < 0.001$).

Among the 78 neonates screened, 51 received oxygen therapy, of whom 15 (29.41%) developed ROP. However, babies who did not require oxygen, did not develop ROP which is in contrast to our study [41]. The difference may be due to the larger sample size in our study. Patel et al. (2019) found that out of 135 cases who required oxygen supplement for ≤3 days, 11 cases were positive for ROP, while out of 91 cases who required A significant association was observed between oxygen therapy lasting more than three days and the development of ROP, with 58 cases testing positive. This correlation was statistically significant ($p < 0.001$, :79.195) [42] Patil et al. (1997) in their prospective study (1997) found a significant higher probability (OR 14) of developing ROP in ventilated babies compared to non-ventilated babies [43].

Out of 15 babies who developed RDS and required surfactant, 11(73.3%) developed ROP. Thus, there is very high significant correlation between RDS and ROP (p-value<0.001). The result is comparable to the study done by Patel et al. (2019) in which multivariate logistic regression model showed Odds ratio 3.6 (95% CI 1.35–9.71) (p-value <0.001) [42] and according to Kumar et al. (2011), stepwise logistic regression analysis revealed that respiratory distress syndrome was strongly associated with severe ROP, showing an adjusted odds ratio of 8.1 (95% CI: 2.6–25.1; $p < 0.001$) [10]. In our study, out of 16 babies who had Apnea of Prematurity, 14 developed ROP (87.5%). Apnea was found as significant risk factor on univariate analysis ($p <0.001$). This was also found by Chaudhari et al. (2009) in which univariate analysis showed Odds ratio 5.19 (95% CI 2.91—9.23) and multivariate analysis showed Odds ratio 3.75 (95% CI 1.98–7.09) (p-value -0.001) [5]. Aggarwal et al. (2002) showed multivariate analysis adjusted RR 12.5 (95% CI 3—50.9) i.e. Apnea of Prematurity was a significant risk factor for ROP [11].

In our study, out of 12 babies who received any of the blood products transfusion, 10 developed ROP (83.33%). Therefore, blood transfusion emerged as a highly significant risk factor for the development of ROP ($p < 0.001$). This was also found in the study conducted by Patel et al. (2019) in which multivariate logistic regression model showed Odds ratio 5.734 (95% CI 2.024–16.245) (p-value <0.001) [42] and in the study conducted by Kumar et al. (2011) in which univariate analysis showed Odds ratio 5.19 (95% CI 2.91—9.23) [10]. Maheshwari et al. (1996) in their study found Blood Transfusion as an independent risk factor for development of ROP [44].

In the current study, 25 out of 32 infants diagnosed with sepsis (78.1%) developed ROP, indicating a strong and statistically significant association ($p < 0.001$). Similar findings were reported by Chaudhari et al. (2009), where univariate analysis yielded an odds ratio of 2.17 (95% CI: 1.29–3.66), and multivariate analysis showed an odds ratio of 3.13 (95% CI: 1.56–6.29), with a p-value of 0.001 [5]. Aggarwal et al. (2002) in univariate analysis showed Odds ratio 5.5 (95% CI 2.7-11.4) (p-value <0.001) [11]. Clinical sepsis was an important risk factor even in the study done by Maheshwari et al. (1996) [44]. In our study, among the 11 cases who developed feed intolerance, 9 cases developed ROP (81.8%). Thus, Feed Intolerance was found to be a significant risk factor ($p<0.001$).

Out of 83 twin babies 17 babies (20.48%) developed ROP whereas out of 94 single babies 36 babies (38.3%) developed ROP. In the present study, singleton pregnancy was found to be a significant risk factor for the development of ROP ($p < 0.001$). This finding contrasts with the results of Patel et al. (2019), who identified multiple births as a significant risk factor through multivariate logistic regression, reporting an odds ratio of 3.324 (95% CI: 1.3–8.499; $p < 0.012$) [42]. On the other hand, the study by Nikhil et al. (2016) reported no significant association between the type of gestation and the occurrence of ROP ($p = 0.789$) [41]. In our study, the Singleton pregnancy cases had relatively more risk factors as compared to Twin pregnancy like mechanical ventilation (10 Vs 4), CPAP (39 Vs 20),Nasal Prong (43 Vs 20), Blood Transfusion (8 Vs 4), Sepsis (20 Vs 12), Apnea of Prematurity (10 Vs 6), RDS (11 Vs 5), 28-30 week GA group (14 Vs 2), BW 750-1000 gm group (5 vs 2) and in the BW 1000-1250 gm group (11 Vs 7).Hence the increased incidence of ROP in Singleton Pregnancy in our study.

Out of 125 LSCS deliveries 34 babies (27.2%) developed ROP whereas out of 52 Vaginal Deliveries 19 babies (36.5%) developed ROP. The association between mode of delivery and ROP is considered to be statistically insignificant (p-value -0.217) in the present study which is in contrast to the study conducted by Selvakumar et al. (2018) in which 18 (15.7%) out of 114 LSCS had developed ROP whereas 11 (6.9%) out of 158 Vaginal deliveries had develop ROP (p value -0.038), : 4.324) [45]. In our study number of cases were relatively less compared to the number of cases studied by Selvakumar et al. and hence the difference. In the present study out of 63 cases on phototherapy, 35 developed ROP (55%) [45]. Thus, Phototherapy emerged

as independent significant risk factor on univariate analysis (p <0.001). This was also found in the study conducted by Patel et al. (2019) in which multivariate logistic regression model showed Odds ratio 13.792 (95% CI 2.798-67.992) (p-value <0.001) [42]. In our study ,4 cases had convulsion and 2 of them developed ROP (50%).

The association between convulsion and ROP is considered to be statistically insignificant (p-value -0.376). This is in contrast to the study conducted by Patel et.al (2019) in which neonatal seizure was found as a significant risk factor on univariate analysis [42]. A greater number of premature neonates who develop convulsion need to be studied for determining their association for ROP. Of the 2 cases who had IVH in our study, only 1 (50%) developed ROP. The association between Intraventricular Haemorrhage and ROP is considered to be statistically insignificant (p-value-0.491) in the present study This was also found in the study conducted by Patel et.al (2019) who studied 286 cases in 1 year (2017-18) (p-value >0.05) [42]. However, it is in contrast to the study conducted by Kumar et al. (2011) in which univariate logistic regression of Intraventricular Haemorrhage for severe ROP showed Odds ratio 7.7 (95% CI 3.2-18.7) p<0.001 which was significant [10]. The duration of study of Kumar et al. (2011) is of 5 years (2003-2008) where 704 cases were studied and hence a greater number of IVH cases were picked up whereas our study duration was 1 year (1.9.2019 to 31.08.2020) and the sample size was smaller (177), hence less cases of IVH were diagnosed. The more the number of cases the better the prediction of association of risk factor.

In the present study, babies with PDA that was hemodynamically significant and required treatment were only 2 and none developed ROP. But this was not significant on analysis due to small number which is in contrast to the study conducted by Kumar et al. (2011) in which univariate logistic regression of PDA that was hemodynamically significant and required treatment for severe ROP showed Odds ratio 9.1 (95% CI 4.3-19.5); p<0.001 where the number of cases studied were 704 and their study duration was for 5 years [10].

A one-year, hospital-based prospective study was carried out at the NICU of Kurji Holy Family Hospital, a tertiary care center, to determine the incidence of ROP and identify its associated risk factors. A total of 177 neonates met the eligibility criteria for inclusion in the study. The incidence of ROP was 29.94%. Lower Birth Weight was significantly associated with increased incidence. Low Gestational Age is an independent risk factor for ROP. The incidence of ROP showed a significant inverse relationship with both birth weight and gestational age ($p < 0.001$). Univariate analysis identified several significant risk factors, including prematurity, low birth weight, oxygen supplementation, respiratory distress syndrome (RDS), apnea of prematurity, blood transfusion, sepsis, feed intolerance, phototherapy (all with $p < 0.001$), and singleton pregnancy ($p < 0.01$). Multivariate analysis using stepwise logistic regression highlighted gestational age below 34 weeks ($p < 0.001$), birth weight under 1250 grams ($p < 0.001$), use of CPAP ($p < 0.001$), blood transfusion ($p = 0.017$), and RDS ($p = 0.093$) as independent predictors of ROP.

This study underscores the significant burden of ROP among preterm infants. With improved survival of smaller neonates, the incidence of ROP may continue to rise unless contributing risk factors are simultaneously addressed. In developing countries like India, all preterm ≤34 weeks should be screened irrespective of risk factors, but with associated risk factors like mechanical ventilation, oxygen therapy, blood transfusion, sepsis and feed intolerance, even babies with up to 36 weeks and weight up to 2000gm should be screened. Oxygen therapy in newborns should be administered with caution, aiming to minimize its duration whenever possible. Similarly, the use of blood products must be carefully considered. Preventing preterm births, reducing exposure to known risk factors, and ensuring careful management of critically ill neonates can collectively help lower the incidence of ROP. Effective screening and timely intervention will prevent the progression of ROP to end stages thus decreasing the incidence of Blindness in children.

Limitation of the study

A larger multi-centric study over a longer duration of period is required to establish the true incidence and causal relationship of risk factors associated with ROP in a developing country like India. Lack of long term follow up to assess future ophthalmological sequelae including myopia, cataract, squint and other long-term complications associated to ROP. More cases of Prematurity with Convulsion, Intraventricular Hemorrhage and significant PDA need to be studied to find their association with ROP.

Abbreviations

ROP – Retinopathy of Prematurity GA – Gestational Age AAP – American Academy of Paediatrics IAP – Indian Academy of Paediatrics VEGF – Vascular Endothelial Growth Factor

ICROP – International Classification of Retinopathy Of Prematurity

RLF – Retrolental Fibroplasia

ETROP – Early Treatment For ROP

NICU – Neonatal Intensive Care Unit

APROP – Aggressive Posterior Retinopathy of Prematurity

IVH – Intraventricular Haemorrhage

RDS – Respiratory Distress Syndrome

CLD – Chronic Lung Disease

PDA – Patent Ductus Arteriosus

O2 – OXYGEN

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Psychometric Validation of the Kazakh Version of the 9-Item European Heart Failure Self-Care Behaviour Scale

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ABSTRACT

Introduction: Heart failure (HF) remains a leading global health challenge, with high hospitalization and mortality rates. Beyond pharmacological and device-based treatment, patient self-care is essential. The European Heart Failure Self-care Behaviour Scale (EHFScBS-9) is widely used to assess self-care, but no validated Kazakh version exists. This study aimed to translate, culturally adapt, and psychometrically validate the EHFScBS-9 in patients with chronic HF.

Methods: Translation followed the Brislin model with three independent forward translations, focus group review, and back-translation by a professional translator unfamiliar with the original instrument. The back-translation was reviewed and approved by the author of the scale, Professor T. Jaarsma (Linköping University, Sweden). A total of 78 patients with chronic HF hospitalized in the cardiology department between December 2024 and June 2025 were enrolled. Exclusion criteria were cognitive impairment (Mini-Cog <3) and non-Kazakh speakers. Ethical approval and informed consent were obtained. Participants completed both the Kazakh EHFScBS-9 and the Kansas City Cardiomyopathy Questionnaire (KCCQ). Diagnosis and management of HF were performed in accordance with the clinical guidelines of the Ministry of Health of the Republic of Kazakhstan. Internal consistency was evaluated using Cronbach's alpha, construct validity by factor analysis, and discriminant validity by Spearman's correlation with the KCCQ.

Results: mean age of participants was 64.5 years (28–88), with 63.3% males. The mean left ventricular ejection fraction was 40.3 ± 7.2%. The average EHFScBS-9 score was 25.9 ± 9.1, while the mean KCCQ score was 51.6 ± 14.6. The Kazakh EHFScBS-9 showed good internal consistency (Cronbach's alpha = 0.82). Factor analysis supported the scale's construct validity. A moderate negative correlation with the KCCQ (r = -0.47; p < 0.001) confirmed discriminant validity.

Conclusion: The Kazakh version of the EHFScBS-9 is reliable and valid for assessing self-care in patients with chronic HF, making it suitable for clinical practice and research in Kazakhstan.

Keywords: Heart Failure; Kazakhstan; Psychometric; Self Care; Surveys and Questionnaires.

Introduction

Heart failure (HF) is a chronic and progressive clinical syndrome and represents the terminal stage of the most common cardiovascular disorders, such as hypertension and coronary artery disease [1]. According to global epidemiological studies, HF affects over 60 million individuals worldwide and its prevalence is projected to rise further due to population aging, enhanced post-acute cardiovascular events survival, and the widespread use of life-prolonging evidence-based therapies [2]. Despite progress in early detection and treatment, HF continues to be one of the major cause of mortality and functional impairment [3]. As a growing public health concern, chronic heart failure (HF) significantly strains medical resources while deteriorating patients' lives, characterized by its epidemic

proportions, repeated hospital readmissions due to frequent episodes of decompensation, and unfavorable survival rates [4]. Patients' daily lives are profoundly affected, as persistent dyspnea, fatigue, limited physical activity, and dependence on medical care lead to reduced quality of life and diminished social engagement in individuals with HF [5,6]. HF is associated with 1-year post-hospitalization mortality rates ranging from 17% to 45%, highlighting the need for comprehensive management strategies [2]. Patient self-care and self-management behaviors are recognized as key components of this approach [7]. Strong associations exist between effective self-care behaviors and superior clinical outcomes in HF, including better quality of life, decline in hospital readmissions, and lower mortality [8,9]. However, in clinical practice, the patients' self-care performance is often suboptimal [10]. Across countries, only 6.9% to 48% of patients demonstrate sufficient adherence to recommended behaviors [11]. Self-management is considered a modifiable determinant, amenable to improvement through targeted educational and behavioral interventions [12]. Consequently, self-care assessment serves as a valuable clinical tool for identifying patients who require additional support and targeted interventions [13].

Self-care assessment is of particular importance in countries with a high cardiovascular disease burden, including Kazakhstan, where HF remains a significant clinical and social issue [14]. Effective management requires strengthening patients` ability to sustain self-care skills, in line with international cardiology guidelines [15]. According to contemporary conceptual models of HF self-care encompasses three interrelated components: maintenance (adherence to therapy and lifestyle recommendations), monitoring (symptom recognition), and management (timely response to deterioration) [12]. Several scales have been developed and validated for the quantitative assessment of self-care [16]. Among various assessment tools, the nine-item European Heart Failure Self-care Behaviour Scale (EHFScBS-9) represents the most frequently utilized measure of self-management capacity in HF patients internationally, demonstrating high reliability and validity [17]. The tool has been translated and adapted into over 20 languages, is endorsed by the European Society of Cardiology (ESC), and has been successfully applied across diverse patient populations. It captures key aspects of daily self-care and enables the identification of skill deficits requiring focused interventions. The development of a Kazakh version of the EHFScBS-9 is essential for both clinical practice and scientific studies in HF. A reliable, validated self-care assessment tool in the national language will enable objective assessment of patient behavior, identification of educational needs, and the design of effective interventions to achieve better health outcomes and life satisfaction among individuals with HF in Kazakhstan.

The objective of this work was to translate, culturally adapt, and assess internal consistency, construct validity, and discriminant validity of the Kazakh translation of the nine-item European Heart Failure Self-care Behaviour Scale (EHFScBS-9) in patients with chronic HF.

Methods

This was a single-center, observational study. Ethical approval was obtained from the Local Ethics Committee of the West Kazakhstan Marat Ospanov Medical University (№ 11.20/03, dated 29.11. 2024).

2.1 Study population

Participants were consecutively recruited from the cardiology department between December 2024 and June 2025. Inclusion criteria were: clinically confirmed diagnosis of HF, age over 18 years, fluent in Kazakh, and achievement of clinical stabilization during ongoing therapy.

Exclusion criteria included significant cognitive impairment as indicated by a Mini-Cog score <3, pregnancy, and estimated life expectancy not exceeding three months, and refusal to participate in the study. Life expectancy greater than three months was determined by the attending physician based on the patient's clinical condition, functional status, comorbidities, and overall disease course.

Data collection occurred in two distinct phases: during the index hospitalization and at a 30- day follow-up. Patient evaluation included assessment of symptoms, detailed medical and life history (covering marital status, education, lifestyle risk factors, and comorbidities), physical examination, laboratory and instrumental investigations, a prescription of therapy in accordance with the clinical protocol for the diagnosis and management of chronic HF of the Republic of Kazakhstan (Protocol No. 179, dated February 9, 2023). Participants were administered two validated instruments: the KCCQ for disease-specific quality of life assessment and the EHFScBS-9 for self-care behavior evaluation. Patients completed the questionnaires independently under the supervision of trained staff in one-on-one sessions held in a dedicated room.

2.2 Questionnaire

EHFScBS-9

The nine-item EHFScBS quantifies self-care behaviors across three conceptual domains: maintenance, monitoring, and management. Each item is answered on the five-point Likert scale: from 1 — "I completely agree" to 5 — "I do not agree at all". Final scores (range: 9-45) are obtained by summing responses to all nine questions. Coding is inverse, with 9 representing ideal self-care[17].

The Kansas City Cardiomyopathy Questionnaire (KCCQ)

The KCCQ is a HF-specific tool contains 23 questions grouped into six domains: Physical limitations, Symptom frequency and severity, Symptom stability, Self-efficacy and knowledge, and Social limitation. Scores range from 0 to 100, with higher values indicating better health status and quality of life. The KCCQ is highly sensitive to changes in a patient's condition and is widely used in both clinical research and practice[18].

Clinical Condition Assessment Scale for chronic heart failure

This tool, modified by Professor V.Yu. Mareev, evaluates the severity of clinical manifestations of chronic HF. Scores range from 0 (no HF) to 20 (end-stage HF). According to this scale, functional classes correspond to the following scores: Class I — ≤3 points; Class II — 4–6 points; Class III — 7–9 points; Class IV — >9 points.

2.3 Translation and Adaptation Process of the EHFScBS-9 into Kazakh

The Kazakh version was adapted from the English EHFScBS-9 using established translation methodologies. The EHFScBS-9 was translated into Kazakh following Brislin's model through these steps:

European Heart Failure Self-Care Behavior Scale_9

Жүрек жеткіліксіздігі бар науқастардың өзіне-өзі көмек көрсету қабілетін бағалаудың еуропалық шкаласы

Бұл шәкіл (шкала) жүрек жеткіліксіздігі бар адамдардың өзін-өзі бақылауына байланысты тұжырымдардан тұрады. Әрбір тұжырымға жауап беріп, сіздің ойыңызша өзіңізге көбірек сәйкес келетін санды қоршап қойыңыз. Жауаптар «Толығымен келісемін» (1) бастап «Мүлдем келіспеймін» (5) деген аралықтағы шәкілден тұратындығына назар аударыңыз. Егер сіз қандай да болсын тұжырымға жауап беруге күмәнданатын болсаңыз да, сіз үшін ең лайықты болып табылатын санды қоршап қойыңыз.

	Толық келісемін				Толық келіспеймін
1. Мен салмағымды күнде өлшеймін	1	2	3	4	5
2. Егер менің ентігуім күшейсе, мен дәрігерге немесе медбикеге хабарласамын	1	2	3	4	5
3. Егер менің аяқбасы/аяқтарымның ісінуі күшейсе, мен дәрігерге немесе медбикеге хабарласамын	1	2	3	4	5
4. Егер менің салмағым аптасына 2 кг-нан артық өссе, мен дәрігерге немесе медбикеге хабарласамын	1	2	3	4	5
5. Мен ішетін сұйықтықтың мөлшерін шектеймін(тәулігіне 1,5-2 л артық емес)	1	2	3	4	5
6. Егер шаршау үдейген кезде, мен дәрігерге немесе медбикеге хабарласамын	1	2	3	4	5
7. Мен тұзы аз диетаны ұстанамын	1	2	3	4	5
8. Мен дәрі-дәрмектерді тағайындағандай қабылдаймын	1	2	3	4	5
9. Мен физикалық белсенділіктің ұсынылған деңгейін қолданамын	1	2	3	4	5

The European Heart Failure Self-care Behavior Scale (Jaarsma, Stromberg, Martensson, Dracup, 1999)

Figure 1 – The Kazakh adaptation of the EHFScBS-9 questionnaire

1. Forward Translation: Performed independently by 2 cardiologists fluent in English and 1 professional translator without medical background

2. Expert Consensus: A focus group discussed all three translation variants, approved each scale item through formal voting

3. Back Translation conducted by a professional translator (unfamiliar with the original scale) from a certified translation agency

4. Approval by the original author of the scale

The final draft of the instrument was reviewed and approved by the original author, Professor T. Jaarsma (Linköping University, Sweden), who endorsed the translation and published it on the university's website https://liu.se/en/research/european-heart-failure-self-care-behaviour-scale/ehfscb-versioner

The original English version of the EHFScBS-9 scale is provided in the Appendix (Supplementary Figure S1), while the adapted Kazakh version is presented in Figure 1.

2.4 Statistical analysis

Data were analyzed using IBM SPSS Statistics for Windows (IBM Corp., Armonk, NY, USA, v. 25).

Quantitative variables were summarized using mean, standard deviation, median, and interquartile range according to their distribution, while categorical variables were reported as percentages. The internal consistency of the Kazakh translation of EHFScBS-9 was assessed by Cronbach's alpha, which reflects agreement among items in the scale. Construct validity was examined through factor analysis using principal component analysis (PCA) with orthogonal rotation (varimax method). Discriminant validity of the 9-EHFScBS was evaluated using Spearman's correlation analysis between self-care scores and KCCQ total scores. Statistical significance was defined as $p < 0.05$.

Table 1 Demographic and clinical characteristics of the study participants

Characteristics	Percentage (n=78)
Age, years	64,5 (28-88)
Sex	
Male, n(%)	50 (64,1%)
Female, n(%)	28 (35,9%)
Marital status	
Married/living with a partner or family, n(%)	60 (76,9%)
Single, n(%)	18 (23,1%)
Education	
None or primary, n(%)	9 (11,5%)
Secondary, n(%)	52 (66,7%)
Higher, n(%)	17 (21,8%)
Smoking	
Yes, n(%)	15 (19,2%)
No, n(%)	63 (80,8%)
Body Mass Index, g/m^2	29,0 (18-47,4)
Etiology of chronic heart failure	
Ischemic, n(%)	48 (61,5%)
Non-ischemic, n(%)	30 (38,5%)
Left ventricular ejection fraction (%),(mean ± standard deviation)	40,3± 7,2
NYHA class	
II, n(%)	1 (1,3%)
III, n(%)	46 (58,2%)
IV , n(%)	27 (34,2%)
Sinus rhythm, n(%)	43 (55,8%)
Atrial fibrillation, n(%)	34 (44,2%)
Systolic Blood Pressure, mmHg	140±25,9
Heart Rate, bpm	95± 18,9
Glomerular Filtration Rate, ml/min/1,73m2	74,3±22,2
proBNP pg/ml	5252 (115-30300)
Comorbidities	
Diabetes Mellitus, n(%)	32 (41%)
BMI>30кг/м2, n(%)	19 (24,4%)
eGFR < 60 мл/мин, n(%)	17 (21,8%)
Anemia, n(%)	6 (7,7%)
ICD/ CRT-D, n(%)	7 (9)%
Stenting / CABG, n(%)	20 (25,6%)
Medications	
ACEI/ARB, n(%)	73 (93,6%)
Beta-blockers, n(%)	74 (94,9%)
Mineralocorticoid Receptor Antagonists, n(%)	70 (89,7%)
Sodium-Glucose Co-Transporter,n(%)	62 (80,5%)
Diuretics, n(%)	68 (87,2%)
EHFScBS-9 questionnaire, points	25,9±9,1
Quality of life (KCCQ), %	51,6±14,7

ACEI/ARB — angiotensin converting enzyme inhibitors/angiotensin receptor blocker;
CABG- Coronary Artery Bypass Grafting
CRT-D - Cardiac Resynchronization Therapy with Defibrillation
ICD- Implantable Cardioverter-Defibrillator
NYHA — New York Heart Association
proBNP - pro–brain natriuretic peptide

Results

The research involved 50 male and 28 female participants, aged 28–88 years. A large proportion of patients were classified as New York Heart Association (NYHA) class III HF. Most patients were in a relationship or lived with a family (76,9%) and had secondary education (66,7%). 19,2% of participants reported current smoking. The etiology of HF among the participants was predominantly ischemic, primarily attributed to coronary artery disease in 61,5% of cases, whereas non-ischemic causes accounted for the remaining 38,5%. Most patients had comorbid diabetes mellitus (41%) and chronic kidney disease (21,8%). Table 1 presents detailed characteristics of the patient population.

Test–retest reliability was assessed by re-administering the questionnaire one month later with good agreement across scales, as illustrated in Figure 2.

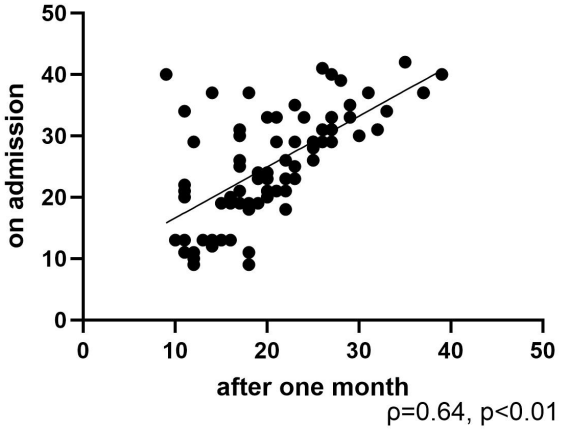

Figure 2 – Correlation between total EHFScBS-9 scores on admission and after one month

The scatterplot illustrates the correlation between total EHFScBS-9 scores at baseline and after one month, indicating good reproducibility and reliability (Spearman's ρ=0.64, p<0.01).

The Kazakh version of the EHFScBS-9 demonstrated a Cronbach's alpha of 0.82, values ranged from 0.79 to 0.83 when individual items were excluded, as shown in Table 2.

Table 2 presents the internal consistency analysis of the Kazakh version of the EHFScBS-9. Corrected item–total correlations ranged from 0.36 to 0.69, indicating acceptable associations between individual items and the overall scale. The values of "Cronbach's alpha if item deleted" (0.79–0.83) show that removal of any item would not substantially improve the reliability of the scale. These findings support the adequate internal consistency of all nine items.

Construct validity was assessed using principal component analysis (PCA). Table 3 presents standardized factor loadings for the questionnaire items, with coefficients greater than 0.30.

Table 3 summarizes the factor structure of the Kazakh version of the EHFScBS-9. PCA identified three distinct components.

A statistically significant negative correlation was found between the Kazakh version EHFScBS-9 and KCCQ scores (r=−0.47, p<0.001), as shown in Figure 3.

The figure illustrates inverse correlation between EHFScBS-9 and KCCQ total scores. Higher self-care ability (lower EHFScBS-9 scores) corresponded to higher health-associated quality of life (KCCQ), supporting discriminant validity of Kazakh adaptation of the scale.

Discussion

This study evaluated the psychometric properties of the Kazakh version of the EHFScBS-9, providing the first validation of a self-care assessment tool for patients with chronic HF in Kazakhstan. The finding confirm that the scale demonstrates good reliability and validity in this population.

Currently, only two reliable and validated tools exist to specifically assess self-care in chronic HF: the Self-Care Heart Failure Index (SCHFI) and the European Heart Failure Self-Care Behavior Scale (EHFScBS)[18]. EHFScBS-9 was chosen due to its relevance to clinical practice, user-friendly structure, and effectiveness in both academic and routine applications.

Based on study results, the Kazakh version of EHFScBS-9 indicated strong homogeneity among items, with a Cronbach's α= 0.82. Temporal stability was assessed after a one-month interval and indicated a satisfactory level of stability. Factor analysis supported the conceptual division of the scale into three domains: maintenance, monitoring, and management. Convergent validity analysis revealed an inverse correlation

Table 2 Internal Consistency Reliability of the EHFScBS-9 Questionnaire

Variable	Scale Mean if Item Deleted	Scale Variance if Item Deleted	Corrected Item-Total Correlation	Squared Multiple Correlation	Cronbach's Alpha if Item Deleted
1 I weigh myself every day	22,33	65,99	0,51	0,30	0,81
2 If my shortness of breath increases, I contact my doctor or nurse	23,90	67,13	0,56	0,84	0,80
3 If my feet/legs become more swollen than usual, I contact my doctor or nurse	23,77	66,72	0,53	0,84	0,81
4 If I gain 2 kilos in one week I contact my doctor or nurse	21,99	63,03	0,69	0,59	0,79
5 I limit the amount of fluids I drink (not more than 1½-2 l/ day)	22,82	69,58	0,36	0,33	0,83
6 If I experience increased fatigue I contact my doctor or nurse	22,64	63,92	0,60	0,49	0,80
7 I eat a low salt diet	23,17	67,93	0,45	0,38	0,82
8 I take my medication as prescribed	23,79	66,29	0,59	0,40	0,80
9 I exercise regularly	23,08	68,02	0,48	0,40	0,81

Table 3 Factor Structure of the Kazakh Version EHFScBS-9 Questionnaire

Question	Component		
	1	2	3
1 I weigh myself every day	0.631		
2 If my shortness of breath increases, I contact my doctor or nurse		0.941	
3 If my feet/legs become more swollen than usual, I contact my doctor or nurse		0.947	
4 If I gain 2 kilos in one week I contact my doctor or nurse	0.698	0.389	
5 I limit the amount of fluids I drink (not more than 1½-2 l/day)			0.889
6 If I experience increased fatigue I contact my doctor or nurse	0.541	0.436	
7 I eat a low salt diet			0.757
8 I take my medication as prescribed	0.603		
9 I exercise regularly	0.883		

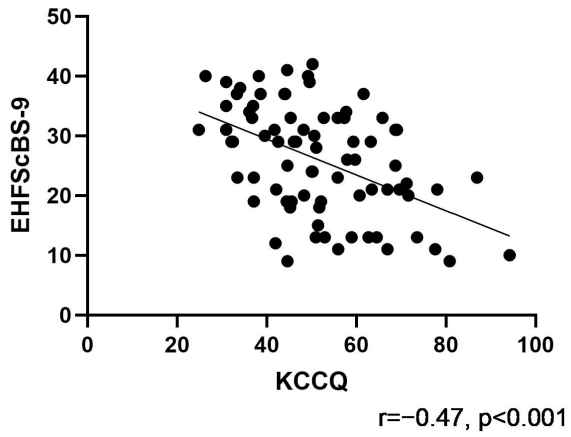

Figure 3 – Correlation between EHFScBS-9 and KCCQ

between the two instruments: improved self-care ability (lower EHFScBS-9 scores) was associated with better quality of life (higher KCCQ scores).

Importantly, the cohort of Kazakh-speaking patients included in our study differs from the populations examined in previous international validation studies in several key aspects. Our inpatients had a high burden of advanced heart failure (NYHA class III–IV), and a high prevalence of comorbidities such as hypertension and type 2 diabetes. In addition, lower levels of health literacy and limited exposure to structured self-care education programs distinguish Kazakh-speaking individuals from more urbanized populations typically studied in Europe and East Asia. In several regions of Kazakhstan, access to structured self-care education remains limited and is largely concentrated in major urban centers. This pattern reflects systemic characteristics of the healthcare infrastructure rather than differences in the general educational attainment of the population.

Psychometric properties of our version of the questionnaire, (α = 0.82), are comparable with the original version (α = 0.80) [17] and with China (α = 0.82) [19], the USA (α = 0.80) validations [20]. Moreover, the Kazakh version showed slightly higher reliability than versions used in Poland (α = 0.78) [21], Italy (α = 0.78) [22], Germany (α = 0.71) [23], Japan (α = 0.71) [24], Brazil (α = 0.70) [25], and the United Kingdom (α = 0.68) [26], although lower than those reported in Korea (α = 0.84) [27].

In addition to internal consistency, the scale demonstrated good test–retest reliability over a one-month interval, with a correlation coefficient of $\rho = 0.64$ ($p < 0.01$), indicating good temporal stability. This suggests that Kazakh translation of the EHFScBS-9 provides stable and reproducible measurements of self-care behavior over time.

Factor analysis did not support the subdivision of the EHFScBS-9 into subscales, indicating that the scale should be used as a unidimensional instrument. The original author, T. Jaarsma, proposed a hypothetical division into three subscales: (1) adherence-related behaviors (items 1, 5, and 9), (2) adaptive responses to symptoms (items 2, 3, 4, and 6), and (3) behaviors dependent on healthcare providers (items 7 and 8) [17]. The weak inverse correlation identified between the EHFScBS-9 and KCCQ in our study confirms that these scales evaluate separate dimensions of heart failure management: behavioral engagement and patient-reported outcomes, respectively.

Engagement in self-care behaviors and self-management recommendations in HF—including medication compliance, weight monitoring, sodium and fluid restriction, regular physical activity, timely medical consultation—represents a set of modifiable factors strongly associated with clinical outcomes and patient well-being.

The validated Kazakh version of the EHFScBS-9 represents a reliable instrument for standardized evaluation of self-care behaviors in HF, particularly within Kazakhstan's multilingual population.

Its application offers several important advantages:

– identification of key self-management deficits, including insufficient symptom recognition, poor compliance with therapy, failure to follow clinical recommendations;

– stratification of patients requiring targeted educational or behavioral support;

– monitoring of self-care dynamics over time, including evaluation of intervention effectiveness.

These capabilities support the personalization of care, more accurate planning of therapeutic and preventive strategies, and ultimately, improved clinical outcomes. Our findings do not imply the need for different pharmacological strategies but emphasize the importance of enhanced patient education and structured self-care support for individuals with advanced HF and multiple comorbidities. The development and implementation of the Kazakh version of the EHFScBS-9 holds significant value for both clinical practice and scientific research. A reliable assessment of self-care will enable the design of targeted educational programs tailored to patient needs, thereby contributing to enhanced quality of life among individuals living with heart failure.

Additional studies are required to evaluate the predictive power of the scale, specifically its ability to forecast clinical outcomes such as rehospitalization or mortality, to assess the scale's sensitivity to changes in patient behavior following

educational programs or other interventions,

to develop normative data and threshold values that allow for more accurate interpretation of individual results in clinical practice, and to confirm the psychometric properties of the scale in larger and more diverse samples, including patients with varying sociocultural backgrounds and comorbid conditions.

Limitations

Patients were recruited exclusively during inpatient treatment, which may have influenced their motivation to demonstrate better self-care and may not fully reflect their behavior in outpatient settings. The single-center design and relatively small sample size may constrain the generalizability of the study results. Multicenter studies involving both inpatients and outpatients are needed to confirm the results and improve the external validity of the scale.

Conclusion

The Kazakh version of the EHFScBS-9 demonstrates satisfactory psychometric characteristics, with strong internal consistency, acceptable reproducibility, and evidence of construct and discriminant validity. These findings support its suitability as a reliable and valid instrument for measuring self-care behavior among chronic HF patients in Kazakhstan. The scale's brevity (9 items) and ease of administration makes it particularly suitable for routine clinical practice and for multicenter clinical and epidemiological studies. Its implementation may facilitate the identification of self-care deficits, enable targeted interventions, and ultimately contribute to improved clinical outcomes and quality of life among patients with HF.

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Logarithm of Thyroid-Stimulating Hormone and Thyroglobulin Product as a Confounder-Adjusted Predictor of Placenta Previa and Placental Abruption

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Abstract

Introduction: Thyroid hormones are essential for placental development, and subtle disruptions may contribute to placenta previa (PP) and placental abruption (PA). This study evaluated thyroid hormone levels and derived log ratios to identify associations with delivery outcomes and predictors of PP/PA.

Methods: In this prospective study, 347 singleton pregnancies at a tertiary obstetric center were included, excluding women with thyroid or chronic disease, smoking, thyroid-affecting medications, or fetal anomalies. Maternal demographic and obstetric data were collected from medical records. Dried blood spot samples were analyzed for thyroid-stimulating hormone (TSH), total thyroxine (TT4), and thyroglobulin (Tg), and urinary iodine concentration (UIC) was measured. Derived thyroid hormone log ratios—including TSH/TT4, TT4/Tg, TSH·Tg, UIC/TT4 were calculated.

Results: Correlations with PP/PA were: TSH (0.50 mU/L; r = –0.123, p = 0.022), Tg (9.42 µg/L; r = –0.119, p = 0.027), log(TSH/TT4) (–2.359 ± 0.283; r = –0.168, p = 0.002), log(TT4/Tg) (1.063±0.366; r = 0.187, p = 0.0005), log(TSH·Tg) (0.574±0.511; r = –0.216, p < 0.001). Logistic regression: log(TSH·Tg) [OR (odds ratio) = 0.16, p = 0.016; log(UIC/TT4) OR = 0.01, p = 0.055. Receiver operating characteristic analysis showed area under the curve 0.857, sensitivity/specificity pair = 100%/61.2% at cutoff < 0.4958, criterion = 0.4958.

Conclusions: Log(TSH·Tg) is a strong independent predictor of PP/PA with high sensitivity and moderate specificity. It outperforms individual thyroid measures and may support early risk stratification by reflecting subtle maternal thyroid dysregulation affecting placental development.

Keywords: Logarithm of thyroid hormones; adjusted predictor for Placenta Previa; Placental Abruption; Thyroid derived ratios; Thyroid-Stimulating Hormone.

Introduction

Thyroid hormones play a pivotal role in maintaining maternal–fetal homeostasis during pregnancy, regulating cellular differentiation, energy metabolism, vascular remodeling, and placental development [1]. Even subtle disturbances in thyroid function may adversely affect placental implantation, trophoblastic invasion, and uteroplacental perfusion, thereby contributing to complications such as placenta previa (PP) and placental abruption (PA) [2]. These

conditions represent major obstetric emergencies associated with significant maternal hemorrhage, fetal distress, preterm birth, and even life-threatening outcomes for both mother and infant [3]. Traditionally, maternal thyroid function has been evaluated through isolated measurements of thyroid-stimulating hormone (TSH) and total thyroxine (TT4); however, these parameters alone may not fully capture the complex interactions within the hypothalamic–pituitary–thyroid axis or reflect peripheral tissue responsiveness [4].

Recently, the use of derived thyroid hormone ratios and composite indices has emerged as a more integrative approach to assessing thyroid homeostasis and its downstream effects on maternal and placental physiology [5-7]. Derived ratios and indices, such as TSH/TT4, TT4/thyroglobulin (Tg), TSH · Tg, urinary iodine concentration (UIC)/TT4, TSH/body mass index (BMI), and TSH composite index, provide a multidimensional view of thyroid function by reflecting both pituitary feedback and peripheral thyroid hormone activity [8].

We hypothesize that using the TSH Index as the main composite measure, together with other derived thyroid hormone ratios, may allow more precise quantification of subtle deviations in thyroid homeostasis and could improve the prediction of adverse placental outcomes such as PP and PA, given the currently limited research on the impact of these thyroid-derived ratios and indices on such complications [9].

The objectives of this study are to assess the levels of thyroid hormones and derived indices in the selected cohort of parturients, to evaluate their correlations with delivery outcomes in women with and without PP or PA, and to identify the strongest predictors of these placental complications. Additionally, we aim to determine the main predictor and establish its optimal cutoff value for predicting the occurrence of these adverse placental events during delivery.

Methods

Study Population

This prospective study included 347 pregnant women who delivered at the "University Clinic of Gynecology and Obstetrics - Skopje". All participants were singleton pregnancies at any gestational age, excluding women with known thyroid disorders, abnormal thyroid test results at sampling, chronic systemic diseases (including autoimmune, renal, hepatic, or cardiovascular conditions), diabetes mellitus, hypertensive disorders, smoking, medications affecting thyroid function, or fetal anomalies, to ensure that all participants had normal thyroid function. Maternal demographic, obstetric, and postpartum clinical data, including age, parity, obstetric history, gestational age at delivery, weight, and height, were extracted from medical records. Written informed consent was obtained from all participants, and the study protocol was approved by the Ethics Committee at JZU Clinical Hospital "D-r Trifun Panovski" – Bitola, under the protocol number (03-242/3), with approval granted on 15.03.2025.

Laboratory Analysis

Dried blood spot (DBS) samples were collected at the time of recruitment using the standard finger-prick method onto filter paper cards, air-dried for 24 hours, and stored at −20 °C. TSH and TT4 were measured by time-resolved fluoroimmunoassay (GSP 2021-0010; PerkinElmer, Turku, Finland) at University Children's Hospital, Zurich, Switzerland. Tg concentrations were determined using a DBS Tg sandwich ELISA at ETH Zurich. Urinary iodine concentration (UIC) was assessed from spot urine samples using standard laboratory procedures. DBS and UIC measurements were obtained at varying gestational ages, as indicated by the median gestational age of 32.0 weeks and the wide 25th–75th percentile range (22.0–38.0 weeks). In addition to these measurements, derived thyroid function indices were calculated, including TSH/TT4, TT4/Tg, TSH · Tg, UIC/TT4, and TSH/BMI. A confounder-adjusted TSH index was also computed according to the formula: log(TSH · Tg) + log(UIC/TT4) – log(TSH/TT4) which simplifies to: log(Tg · UIC) [10].

Statistical Analysis

All analyses were performed using MedCalc, version 22.002 (MedCalc Software Ltd, Ostend, Belgium). Due to non-normal distributions, descriptive statistics are presented as median and 25th to 75th percentile. Point-biserial correlations were used to assess associations between PP/PA and maternal demographics, thyroid-derived ratios, and their base-10 (common) logarithmically transformed values ($\log_{10}$-transformed). Linear regression with scatter plots was used to visualize continuous relationships. The binary outcome variable (PP/PA) was coded as 1 for the presence of either condition and 0 for their absence. Adjusted logistic regression, using residuals of BMI and maternal age as covariates, was performed to identify independent predictors, with multicollinearity diagnostics and model discrimination reported. Receiver operating characteristic (ROC) curve analysis was used to determine optimal cutoff values for significant predictors, and the area under the curve (AUC) was calculated to evaluate their discriminatory ability. A 3D surface plot illustrated the relationship between log TSH•Tg, log TSH/TT4, and the predicted probability.

Results

Maternal and biochemical characteristics

A total of 347 parturients who delivered 347 healthy neonates were included in the study. There were nine cases of PP/PA, accounting for an overall incidence of 2.29%. The mothers had a median age of 29 years, a median BMI of 26.81 kg/m², TSH level of 0.500 mU/L, TT4 concentration of 101.4 nmol/L, Tg level of 9.417 µg/L, and UIC of 183.67 µg/L. Table 1 presents the values of the derived thyroid hormone ratios and the TSH index, along with their log-transformed values to improve data symmetry. The remaining detailed results, including the biserial correlations between maternal thyroid parameters and PP/PA, are shown in the same table (Table 1, see the next page).

Biserial Correlation Analysis

Table 1 presents the biserial correlations between PP/PA and thyroid hormone parameters, thyroid-derived ratios and indices, and their log-transformed values. Among the primary thyroid markers, TSH and Tg showed significant negative correlations with PP/PA, while TT4/Tg exhibited a positive and highly significant association. Derived indices such as TSH/TT4, TSH/BMI, and the TSH index were significantly and inversely associated with PP/PA. Following logarithmic transformation, the correlations became stronger and more significant, reflecting improved data symmetry and greater discriminatory capacity. The overall trend suggests that lower TSH-dependent indices, indicating a relative hypermetabolic thyroid state, may predispose to an increased risk of PP/PA.

Table 1 Distribution of clinical and thyroid-derived variables, their log-transformed values, and point-biserial correlations with placenta previa and placental abruption

Distribuion of variables			Correlation with PP / PA	
Variable	**Median**	**25th - 75th P**	**r**	**p**
Age (years)	29	25 to 33	0.0761	0.1573
BMI (kg/m2)	26.81	23.445 to 29.740	0.0080	0.8824
TSH (mU/L)	0.500	0.300 to 0.700	-0.1233	0.0216
TT4 (nmol/L)	101.4	82.575 to 123.775	0.0236	0.6613
Tg (µg/L)	9,417	5.532 to 15.234	-0.1190	0.0266
UIC (µg/L)	183.67	110.933 to 269.525	-0.0096	0.8552
Thyroid-derived ratios and indices			Correlation with PP / PA	
Variable	Median	25th - 75th P	r	p
TSH/TT4	0.00457	0.00305 to 0.00684	-0.1128	0.0358
TT4/Tg	10,592	6.432 to 18.551	0.278	0.000
TSH • Tg	4,342	1.966 to 8.352	-0.1015	0.0588
UIC/TT4	1,852	1.036 to 2.873	-0.0338	0.5301
TSH/BMI	0.0178	0.0111 to 0.0247	-0.1195	0.0260
TSH index	3,192	2.884 to 3.512	-0.1296	0.0157
Log-transformed thyroid-derived ratios and indices			Correlation with PP / PA	
Variable	Mean and SD	Min to max	r	p
log (TSH/TT4)	-2.359 ± 0.283	-3.336 to -1.326	-0.1678	0.0017
log (TT4/Tg)	1.063 ± 0.366	-0.0002 to 2.812	0.1873	0.0005
log (TSH • Tg)	0.574 ± 0.511	-1.757 to 1.984	-0.2164	0.0000
log (UIC/TT4)	0.229 ± 0.327	-0.778 to 1.113	0.0032	0.9524
log (TSH/BMI)	-1.789 ± 0.286	-2.667 to -0.865	-0.1592	0.0029
log (TSH index)	0.494 ± 0.072	0.065 to 0.629	-0.1521	0.0046

PP, placenta previa; PA, placental abruption; BMI, body mass index; TSH, thyroid-stimulating hormone; TT4, total thyroxine; Tg, thyroglobulin; UIC, urinary iodine concentration; P, percentiles; Variables indicated as log-transformed (log base 10) are shown as log(TSH/TT4, log(TT4/Tg), log (TSH • Tg), log(UIC/TT4), log(TSH/BMI), and log(TSH index), thyroid-derived ratios and indices and raw variables with their respective units.

Linear regression analysis

Figure 1 presents a scatter plot of log-transformed TSH•Tg values against the occurrence of PP/PA. The solid blue line (R) represents the fitted linear regression model, with the dashed red lines (p) indicating the prediction interval and the dotted orange lines (CI) showing the confidence interval (CI). The diagonal light-blue line (d) serves as a reference for visual interpretation. The heat map represents the density of data points (kernel density estimation), with warmer colors indicating regions of higher observation concentration and cooler colors indicating sparse data regions; it does not reflect correlation strength, which is quantified by the regression parameters and correlation coefficient. The analysis demonstrates a statistically significant inverse correlation (r = -0.22, p < 0.001) between log(TSH•Tg) and the occurrence of PP/PA, suggesting that higher TSH•Tg values may be associated with lower risk (Figure 1).

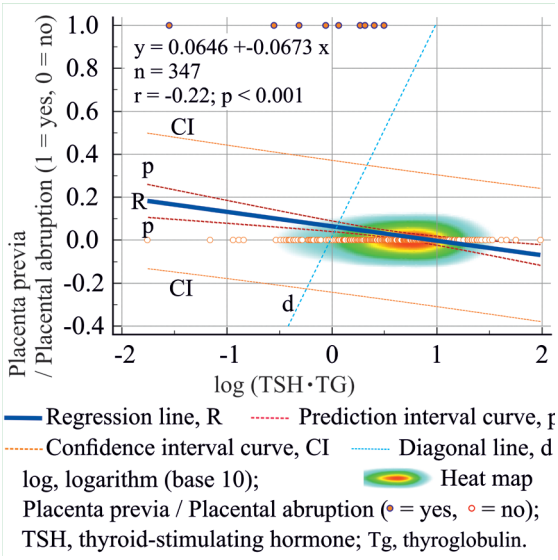


Figure 1 – Scatter plot and linear regression analysis of log (TSH·Tg) against placenta previa/placentalabruption, with heat map illustrating data distribution and areas of maximal density

Logistic regression

In a logistic regression analysis of 347 participants, log (TSH•Tg) was a significant independent predictor of PP/PA (OR = 0.16, 95% CI 0.036–0.71, P = 0.016). Higher log (TSH•Tg) values were associated with a lower risk of these placental events, indicating a protective effect. Specifically, the odds of PP/PA decreased by approximately 84% for each 1-unit increase in log (TSH•Tg). Log (UIC/TT4) showed a trend towards significance (P = 0.055). The high Wald coefficient (5.801) for log(TSH•Tg) indicates its strong predictive power in the model. Age and BMI effects were adjusted using residuals to remove their confounding influence. Variables exhibiting high collinearity, specifically TSH index and log(TSH/BMI), were excluded from the model, resulting in substantially lower variance inflation factors (VIFs) for the remaining predictors

Table 2 Adjusted logistic regression outcomes with multicollinearity diagnostics and model discrimination (ROC analysis)

Logistic regression	
Dependent Y	**Placenta previa / Placental abruption**
Method	Enter
Sample size	347
Positive cases a	9 (2.59%)
Negative cases b	338 (97.41%)
a Placenta previa / Placental abruption = 1	
b Placenta previa / Placental abruption = 0	
Overall Model Fit	
Null model -2 Log Likelihood	83,502
Full model -2 Log Likelihood	58,994
Chi-squared	24,508
DF	6
Significance level	**P = 0.0004**
Cox & Snell R2	0.046819
Nagelkerke R2	0.3188

Multicollinearity diagnostics (VIF)		
Variable	**VIF1**	**VIF2**
log TSH/TT4	13.257	2.018
log (TT4/Tg)	11.101	3.561
log (TSH • Tg)	8.06	5.884
log (UIC/TT4)	8.06	1.216
TSH index	17.076	/
log (TSH/BMI)	15.565	/
ROC curve analysis		
Area under the ROC curve (AUC)		0.857
Standard Error		0.0397
95% CI		0.816 to 0.892
Hosmer & Lemeshow test		
Chi-squared		48,105
DF		8
Significance level		P = 0.777

Coefficients and Standard errors; Odds ratios and 95% Confidence intervals						
Variable	**Coefficient**	**Std. Error**	**Wald**	**Odds ratio**	**95% CI**	**P**
log TSH/TT4	14.86903	9.74225	2.329	2.87•106	0.014 to 562.73•1012	0.127
log (TT4/Tg)	1.35332	2.02022	0.448	3.8703	0.0738 to 202.9481	0.503
log (TSH • Tg)	-1.84081	0.76428	5.801	0.1587	0.0355 to 0.7098	0.016
log (UIC/TT4)	-4.45126	2.32095	3.678	0.0117	0.0001 to 1.1027	0.055
Age REGR Resid1	1.15331	1.17497	0.963	3.1687	0.3168 to 31.6980	0.326
BMI REGR Resid2	-1.38889	1.28016	1.178	0.2494	0.0203 to 3.0656	0.278
Constant	-3.00195	4.58407	0.429	/	/	0.513

DF, degrees of freedom; TSH, thyroid-stimulating hormone; BMI, body mass index; Tg, thyroglobulin; UIC, urinary iodine concentration; TT4, total thyroxine; log, logarithm (base 10); Regr resid1, regression residual. VIF, variance inflation factor; ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval.
Variables indicated as log-transformed (log base 10) include log(TSH/TT4), log(TT4/Tg), log(TSH•Tg), log(UIC/TT4), log(TSH/BMI), and the TSH index, representing thyroid-derived ratios and indices; non-transformed variables are presented as raw values with their respective units.

and confirming improved multicollinearity control. The model demonstrated moderate explanatory power (Nagelkerke R^2 = 0.319), good discriminative ability (AUC = 0.857, 95% CI 0.816–0.892), and adequate calibration (Hosmer–Lemeshow P = 0.777). Full regression coefficients, odds ratios, and CIs are presented in Table 2.

Forest plot

The logistic regression results are additionally summarized in a forest plot (Figure 2), illustrating odds ratios and 95% confidence intervals on a logarithmic scale to enhance comparison of effect sizes and their precision (Figure 2).

Figure 3 presents the receiver operating characteristic (ROC) curve evaluating the diagnostic performance of the log-transformed product of thyroid-stimulating hormone (TSH) and thyroglobulin (Tg) in predicting placenta previa and placental abruption. The ROC curve demonstrates strong discriminative ability, with an area under the curve (AUC) of 0.857 and a statistically significant p-value (P < 0.001). The optimal cutoff for log(TSH•Tg) (< 0.4958) was selected using the Youden index, with intentional prioritization of sensitivity to ensure maximal detection of placental previa/placental abruption cases; this cutoff demonstrates good discriminatory performance in the present cohort and warrants further evaluation in independent populations. These results suggest that log(TSH•Tg) may serve as a promising biomarker for early identification of placental complications (Figure 3).

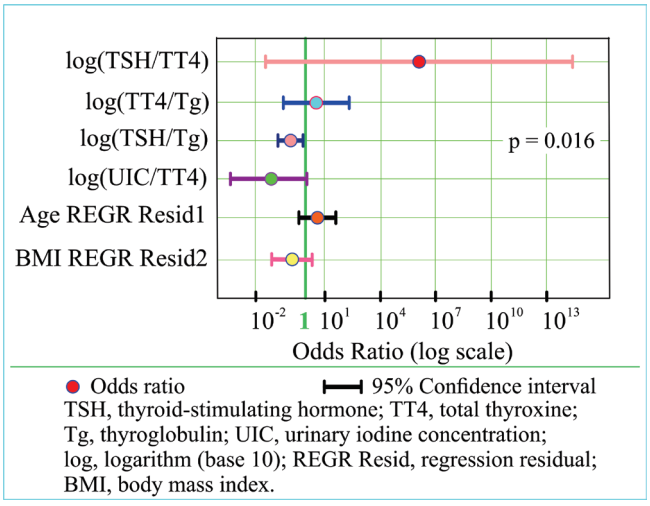
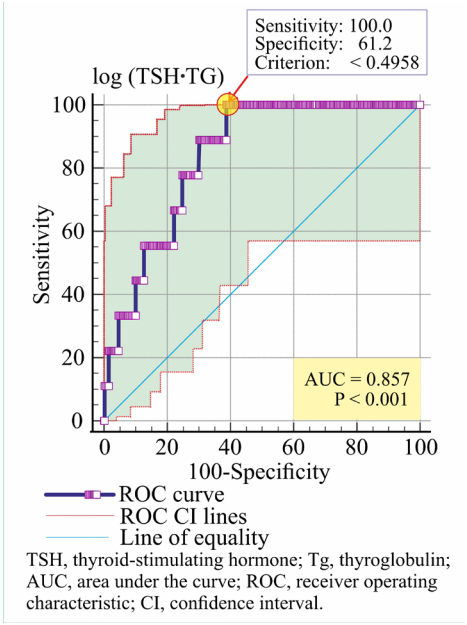


Figure 2 – Forest plot of logistic regression results



TSH, thyroid-stimulating hormone; Tg, thyroglobulin; AUC, area under the curve; ROC, receiver operating characteristic; CI, confidence interval.

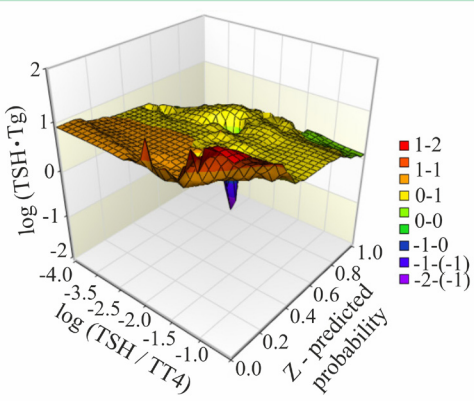
Figure 3 – Receiver Operating Characteristic (ROC) Curve of Log-Transformed (TSH·Tg) for Predicting Placenta Previa and Placental Abruption

Clinical Risk Mapping of Placental Complications Using Thyroid Biomarkers

In Figure 4, a 3D surface plot illustrates the predicted probability of PP/PA as a function of two log-transformed biomarkers: log(TSH/TT4) and log(TSH•Tg). The surface reveals a nonlinear relationship, with varying risk levels across the plot. Color gradients represent different probability zones: warmer colors (red, orange, and yellow) indicate higher predicted risk, while cooler tones (green, blue, purple) reflect lower risk. This visualization highlights how combined thyroid-related markers may help stratify patients by placental complication risk (Figure 4).

Discussion

To our knowledge, this is the first study to investigate the relevance of log-transformed derivatives of thyroid hormone ratios and composite indices for the assessment of PP/PA, providing a novel approach to early risk prediction. Unlike previous work that has relied solely on isolated measurements



TSH, thyroid-stimulating hormone; TT4, total thyroxine; Tg, thyroglobulin.

Figure 4 – Three-dimensional surface plot of the logarithm of TSH · Tg as a function of the logarithm of TSH/TT4 and Z-predicted probability

of TSH, Tg, TT4, and UIC [11, 12], our approach incorporates integrative markers such as log(TSH•Tg), along with a broader panel of derived ratios—including log(TSH/TT4), log(TT4/Tg), log(TSH•Tg), log(UIC/TT4), log(TSH/BMI), the TSH Index, and the composite TSH Index—which together offer a more nuanced assessment of thyroid homeostasis and its possible influence on placental implantation. In this cohort of 347 pregnant women, the observed patterns highlight several endocrine parameters—particularly composite indices—that merit deeper examination within the framework of our study objectives. These considerations provide a foundation for exploring how multidimensional thyroid markers might contribute to the underlying mechanisms associated with placental stability.

In our cohort, the observed incidence of PP/PA was approximately 2.59%, which is generally consistent with rates reported in other obstetric populations. Among the 347 singleton pregnancies included in this study, 9 cases of placental complications were observed (overall incidence 2.59%), comprising 3 cases of PP (0.9%) and 6 cases of PA (1.7%). This distribution aligns with known epidemiology, as PA generally occurs more frequently than PP in the general population (reported incidence ~0.5–1% for abruption vs. 0.3–0.5% for previa) [13]. Although the small number of events limits the ability to calculate precise risk estimates for each complication individually, reporting them separately provides additional context for interpreting our findings. Some studies have reported slightly lower or higher incidences depending on maternal characteristics, parity, and geographic region, but overall our findings align with the expected range [13, 14]. The mothers in our study had BMI and thyroid functional parameters—including TSH, TT4, Tg, and UIC—within ranges typically observed in healthy pregnancies [15, 16]. From these primary measures, we derived a set of ratios and indices, and for further analyses, we focused on their log-transformed values to enhance data symmetry and better capture subtle patterns in thyroid homeostasis.

The biserial correlation analysis provided initial insights into the relationships between maternal thyroid function and the occurrence of PP/PA. Among the primary thyroid markers, TSH and Tg showed significant negative correlations with PP/PA, whereas TT4/Tg exhibited a positive and highly significant association. This pattern suggests that subtle shifts in thyroid homeostasis, particularly those reflecting relative TSH suppression or increased thyroglobulin activity, may be relevant to placental implantation and stability [17] Lower TSH levels are expected in early pregnancy due to β-hCG–mediated stimulation of the maternal thyroid, while both TSH and Tg typically rise in mid-to-late pregnancy as maternal thyroidal demand increases. A combined elevation of TSH and Tg may reflect an adaptive response aimed at maintaining adequate thyroid hormone synthesis to support placental development and function. Physiologically, increased Tg indicates heightened thyroidal stimulation and sufficient iodine substrate, whereas modestly higher TSH values—still within pregnancy-specific reference intervals—help sustain thyroid hormone availability for growing metabolic and fetal needs. These mechanisms align with evidence suggesting that even subtle thyroidal insufficiency, despite biochemical euthyroidism, may impair trophoblast invasion, disrupt endothelial regulation, and predispose to abnormal placentation such as PP and PA [18, 19].

Derived indices, including TSH/TT4, TSH/BMI, and the TSH Index, were inversely correlated with PP/PA, and their logarithmic transformation further strengthened these

associations. The enhanced correlations after log-transformation highlight the value of data normalization in uncovering latent patterns that may not be apparent with raw measurements. Collectively, these findings support the hypothesis that lower TSH-dependent indices—potentially reflecting a relative hypermetabolic thyroid state—could be associated with increased susceptibility to placental complications [20]. While direct comparisons are limited, these observations align with previous reports linking maternal thyroid dysregulation to adverse placental outcomes, reinforcing the need for integrative assessment beyond isolated hormone levels, however, the findings may serve as a useful reference for future research. By controlling for maternal age [21] and BMI [22, 23] using a residuals-based adjustment, the analysis effectively removes their confounding influence, enhancing the precision and robustness of risk prediction.

Clinically, the positive correlation between log transformed TSH•Tg and the risk of PP/PA may reflect subtle but meaningful alterations in the maternal thyroid state that impact placental development [24]. Thyroid hormones are known to regulate trophoblast proliferation, differentiation, and invasion, which are key steps in the formation of a healthy placenta [25]. In particular, dysregulated thyroid signalling can impair the remodelling of maternal spiral arteries and disrupt placental vascular architecture, potentially leading to weak attachment or instability of the placenta. From a mechanistic perspective, the combination of Tg and TSH may serve as a marker of thyroid feedback and hormone production, more closely reflecting the endocrine environment that influences placental development [25].

Higher log(TSH•Tg) could indicate a thyroid milieu that supports more balanced hormone synthesis and secretion, which may favour optimal trophoblast behaviour and placental vascularization. Conversely, lower values might reflect a compensatory dysregulation or overactive feedback loop, which could subtly compromise placental structure and function [26, 27].

In the logistic regression model, log-transformed TSH•Tg emerged as a strong independent predictor of PP/PA, with the risk of PP/PA, with an estimated regression coefficient of -1.841 and a corresponding odds ratio of 0.159. This indicates that for each unit increase in log(TSH•Tg), the odds of developing these placental complications decrease by approximately 84%, highlighting its potential as a protective marker. The high Wald statistic (5.801) underscores the robustness of this predictor in the model. This finding emphasizes the potential relevance of integrative thyroid biomarkers in capturing subtle endocrine alterations that may predispose to placental complications [26]. From a pathophysiological standpoint, the TSH•Tg product reflects both pituitary feedback and thyroid synthetic activity, providing a dynamic measure of maternal thyroid function. Suboptimal or dysregulated thyroid signalling can impair trophoblast proliferation, differentiation, and invasion, as well as spiral artery remodelling, leading to compromised placental attachment and vascular stability [24]. Therefore, lower log(TSH•Tg) values may indicate a thyroid environment less supportive of optimal placental development, whereas higher values may correspond to a more balanced endocrine milieu conducive to healthy placentation. Clinically, this odds ratio provides actionable insight: maternal thyroid indices, particularly log(TSH•Tg), could help identify women at higher risk of placental complications even when conventional hormone levels appear normal. These results support the use of integrative thyroid biomarkers for refined risk stratification in obstetric care, potentially guiding more individualized monitoring or preventive strategies. In other words, women with higher log(TSH•Tg) values have substantially lower chances of developing placental complications, which is consistent with the biological mechanism whereby a balanced thyroid function supports optimal placental vascularization and trophoblast invasion. Given that PP/PA was coded as the presence of placental events, the consistently observed odds ratios below 1 across analyses support a protective association of higher log-transformed thyroid composite indices, particularly log(TSH•Tg).

The 3D surface plot highlights a nonlinear interaction between log(TSH/TT4) and log(TSH•Tg), with color-coded gradients delineating clear low- and high-risk zones for PP/PA. Warmer colors identify biomarker constellations associated with disproportionately elevated predicted risk, supporting the concept that combined thyroid indices better capture subtle endocrine disequilibrium than isolated hormones. This aligns with evidence that even mild thyroid dysfunction may impair trophoblastic invasion and uteroplacental vascular development, thereby increasing susceptibility to placental complications [27].

The ROC-derived cutoff of log(TSH•Tg) < 0.4958, which corresponds to an original (non-log-transformed) TSH•Tg value of approximately < 1.64, effectively identifies women at higher risk. At this threshold, sensitivity is 100%, capturing all true cases, while moderate specificity (61.2%) results in some false positives. These findings confirm log(TSH•Tg) as a robust biomarker for risk stratification, reflecting subtle maternal thyroid dysregulation that may impair trophoblast invasion and placental vascularization.

Strengths and Limitations

The strengths of this study lie in its direct evaluation of the relationship between log(TSH•Tg) and the risk of PP/PA, which is both clinically relevant and pathophysiologically justified. The analysis used residuals to adjust for maternal age and BMI, thereby enhancing the reliability of log(TSH•Tg) as an independent predictor of PP/PA. The use of a log-transformed TSH•Tg index further improves its predictive performance compared with the individual raw thyroid hormone values. Additionally, the study provides a practical clinical threshold (< 0.4958) that can be used for risk stratification and clinical monitoring of high-risk pregnancies.

Limitations of this study include the relatively small sample size and the low number of placental complication events (n = 9; 2.6%), which may limit statistical power, model stability, and the generalizability of the estimated predictive relationships. The moderate specificity of the proposed biomarker may lead to some false positives, and the retrospective or cross-sectional design introduces potential information bias. Additionally, thyroid measurements were obtained across a wide gestational range (22–38 weeks), reflecting the practical constraints of sampling as participants presented during their pregnancies. Although TSH, TT4, and Tg levels vary across trimesters, the single measurements in our cohort are unlikely to have substantially affected the observed associations with PP/PA, as the integrative markers such as log(TSH•Tg) are thought to capture underlying thyroid homeostasis rather than transient trimester-specific fluctuations. While the study applied parsimonious multivariable models and utilized composite indices and logarithmic transformations to enhance signal detection and reduce biological noise, the findings remain

hypothesis-generating rather than definitive. Furthermore, the study focuses on a single biomarker, and the results have not yet been externally validated. Future prospective studies in larger, independent cohorts with a higher incidence of placental complications are warranted to confirm these findings and to explore integration with other clinical risk factors.

Conclusions

Log(TSH•Tg) is a strong independent predictor of PP and PA, even after adjusting for maternal age and BMI. Its high sensitivity allows reliable identification of high-risk pregnancies, though moderate specificity may produce some false positives. Notably, the log (TSH•Tg) ratio index demonstrates greater predictive power than the individual raw thyroid hormone values alone. Clinically, log(TSH•Tg) reflects subtle maternal thyroid dysregulation affecting placental development and can be used for early risk stratification, with positive results interpreted in the broader clinical context to guide appropriate monitoring and management. However, due to the limited number of placental events in this study, these findings should be considered hypothesis-generating and require confirmation in larger, independent cohorts.

Author Contributions:

M. A., the lead physician, oversaw participant selection, study implementation, and medical history analysis. She identified pathophysiological links between bone health and arterial stiffness, supported findings with references, ensured data analysis aligned with results, and critically reviewed the manuscript for scientific rigor. She also conducted a comprehensive literature review and played a key role in the study's conception, data analysis, and discussion.

P. A. contributed to data organization, statistical validation, and ensuring methodological accuracy. He assisted in refining the discussion by integrating relevant findings and enhancing the manuscript's clarity and coherence.

L. T., contributed by analyzing diagnostic imaging, interpreting data, and ensuring accurate result interpretation. She also managed data organization in Excel, enhancing the clarity and reliability of the findings.

B. T. contributed by sourcing relevant literature, refining the discussion, enhancing tables, and improving language, spelling, and grammar.

K. S. applied statistical methods, interpreted results, and provided key insights, ensuring a strong data-driven foundation for the study.

D. Z., an informatics and cloud expert, managed data collection, storage, and processing. He supervised statistical methods and contributed to result interpretation in the discussion. All authors collaborated actively in writing, reviewing, and finalizing the manuscript. All authors collaborated in writing, reviewing, and reaching a unanimous consensus on the final manuscript.

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Data availability statement: The data supporting the findings of this clinical study are included within the manuscript. Due to the sensitive nature of patient's information, additional data will not be made publicly available to maintain patient confidentiality. Specific data requests will be evaluated on a case-by-case basis, with consideration of ethical and privacy requirements.

Patient Informed Consent Statement: Written informed consent was obtained from all participants involved in the study, ensuring they understood the study's purpose, procedures, and their right to confidentiality.

Artificial Intelligence (AI) Disclosure Statement: AI-Unassisted Work.

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Extracellular Bovine-Derived Peritoneum Matrix: Evaluation of a New Biological Implant for Abdominal Wall Reconstruction under Bacterial Contamination in an In Vivo Experimental Model

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ABSTRACT

Introduction: Modern herniology relies heavily on synthetic mesh prostheses, which have markedly reduced recurrence rates and improved outcomes. However, the use of tension-free repair in infected or highly contaminated fields remains insufficiently studied. Biologic implants based on the extracellular matrix (ECM) may attenuate the inflammatory response and reduce the risk of infection. This study evaluates the behavior of different implant types under experimental bacterial contamination.

Methods: Three implants were investigated: an extracellular bovine-derived peritoneum matrix, preserved dura mater (DM), and the composite mesh prosthesis UltraPro. The study was conducted on 78 rats. After the creation of a standardized anterior abdominal wall defect, the defect was repaired using one of the studied implants and subsequently inoculated with MRSA or E. coli. Animals were observed for 10 and 20 days. Microbiological, immunological (circulating immune complexes), hematological, and macroscopic parameters were evaluated.

Results: MRSA contamination induced a pronounced local inflammatory response, including abscess formation on day 10, whereas E. coli was rapidly cleared from the implantation zone. No statistically significant differences in MRSA persistence or semi-quantitative titers were observed between ECM, UltraPro, and DM. Systemically, CIC patterns and hematological changes were similar between ECM and UltraPro, while DM—particularly under MRSA contamination—was associated with a more pronounced inflammatory response.

Conclusions: Under conditions of experimental bacterial contamination, the extracellular bovine-derived peritoneum matrix demonstrated local and systemic inflammatory profiles comparable to those of the synthetic UltraPro mesh and more favorable than those of preserved dura mater. All implants elicited a controlled inflammatory reaction without evidence of systemic septic complications. These findings support further research, including clinical trials, on the use of extracellular bovine-derived peritoneum matrix for reconstructive surgery of anterior abdominal wall defects in contaminated and potentially contaminated fields.

Keywords: biological implant; extracellular matrix; bovine peritoneum; abdominal wall reconstruction; bacterial contamination; MRSA; Escherichia coli.

Introduction

Modern herniology is based on the widespread use of synthetic mesh endoprostheses, which have radically reduced recurrence rates and improved patients' quality of life [1–3]. In recent years, studies have appeared that convincingly demonstrate the feasibility of tension-free techniques in emergency surgery [4, 5]. Tension-free repair is currently recommended for incarcerated hernias [6]. However, no hernia surgery school worldwide provides unambiguous recommendations regarding the use of tension-free repair in highly contaminated wounds, nor regarding the optimal implantation technique and mesh material in such settings [7, 8]. Chronic periprosthetic infection remains one of the unsolved problems of surgery both in Europe and in the USA [9, 10].

Experimental work has shown that under bacterial contamination the reparative process after mesh implantation proceeds more slowly: the phases remain the same but are prolonged approximately twofold [11]. Microbial growth depends on the type and material of the endoprosthesis. It is known that microporous meshes (e.g., polytetrafluoroethylene) should not be used even when there is a minimal risk of infection [12]. The successful use of macroporous mesh endoprostheses (polypropylene) in emergency surgery has been demonstrated in a number of reports [13]. This issue is especially relevant in cases where synthetic materials are used according to relative indications (W1 hernias according to the SWR classification and prophylactic reinforcement) as well as in situations with critically high contamination of the operative field (e.g., peritonitis) [14–16]. The number of studies that comprehensively assess the use of synthetic meshes in a clearly infected operative field remains very limited [17–19].

One of the key problems is the formation of microbial biofilms on the surface of endoprostheses and suture material, which serves as a basis for an unfavorable postoperative course and the development of chronic infection [20, 21, 33]. There are no unambiguous data on the dependence of bacterial growth on the material of macroporous meshes. It has been shown that colonization is associated with hydrophobic properties, multifilament structure, and the presence of niches within the fibers [21]. It has been reliably demonstrated that after tension-free repair in the presence of infection, bacterial contamination of the operative zone persists for at least 90 days, even in the absence of macroscopic signs of inflammation [22]. Thus, the use of mesh endoprostheses and polymer coatings in a compromised field remains an unresolved issue and requires rigorous clinical and experimental investigation.

In contaminated or potentially contaminated surgical fields, the use of permanent synthetic mesh prostheses remains controversial [17-19, 30-34]. Although synthetic meshes provide reliable mechanical reinforcement, they may act as a substrate for bacterial adhesion and biofilm formation, leading to persistent local inflammation and chronic periprosthetic infection. In contrast, biologic implants based on extracellular matrix (ECM) are designed to promote host cell infiltration, neovascularization, and gradual tissue remodeling [31, 32]. These properties may contribute to a more balanced inflammatory response and potentially reduce the risk of long-term infectious complications in conditions of bacterial contamination.

Continuous efforts to search for new plastic materials and to study their behavior in the host organism have led to the development of implants of biological origin. Bioimplants obtained from human donor material (allografts) or animal tissue (xenografts: porcine, bovine) using tissue engineering technologies represent the extracellular matrix (ECM) [23]. The ECM consists of a complex of structural and functional proteins organized into a unique tissue-specific architecture. Beyond its key role as a structural "scaffold", the ECM participates in signal transduction, regulation of cell growth, and differentiation. Biologic materials based on ECM can induce so-called "constructive remodeling", i.e., the formation of functional tissue corresponding to the implantation zone. However, the capacity to induce constructive remodeling strongly depends on the methods used to obtain and process the ECM. Given these functions, ECM-derived implants are widely used in regenerative medicine for tissue reconstruction [24].

The use of such biomaterials in the surgical treatment of anterior abdominal wall hernias holds great promise, as they may minimize the inflammatory response to the implanted prosthesis, reduce the likelihood of adhesions between the abdominal organs and the implant, and lower the risk of wound infection [23].

At the same time, the availability of bioimplants does not resolve a number of issues related to their application in reconstructive surgery. There is no consensus on the indications and optimal technique for biological implant use; long-term outcome data are insufficient; and, importantly, these implants are associated with high cost [25]. Despite a substantial number of publications on xenogenic implants for anterior abdominal wall repair, the findings of different researchers are often contradictory. Moreover, studies specifically investigating extracellular bovine-derived peritoneum matrix (xenoperitoneum ECM) under conditions of bacterial infection are lacking, which underscores the relevance of the present work.

Among various sources of extracellular matrix used for biologic implants, including dermal matrices, small intestinal submucosa, and pericardium, bovine peritoneum represents a thin, collagen-rich tissue with a native peritoneal microarchitecture. After decellularization, this structure can be preserved, potentially facilitating early cellular infiltration, neovascularization, and constructive tissue remodeling. In addition, peritoneum-derived ECM can be manufactured in a standardized manner and tailored specifically for abdominal wall reconstruction, which served as the rationale for selecting xenoperitoneum ECM for evaluation in the present experimental infection model.

The aim of the study: to provide an experimental rationale for the use of xenoperitoneum ECM as a new biological material for the reconstruction of abdominal wall defects of various localizations under conditions of bacterial contamination.

Methods

Object of the study: The object of this comparative experimental study was a novel biological implant developed as a domestic technology and used for the first time for the repair of anterior abdominal wall defects: the xenoperitoneum ECM. The implant was obtained using a double-cycle detergent–enzymatic decellularization protocol, which included treatment with an ionic detergent followed by enzymatic digestion using deoxyribonuclease, and subsequent gamma-ray sterilization [26]. Two implants commonly used in reconstructive surgery served as comparators: preserved dura mater and the composite mesh endoprosthesis UltraPro.

Study design: A comparative experimental study was conducted to assess the microflora and structural changes in the implantation zone of xenoperitoneum ECM under bacterial

contamination. The experiment was performed on 78 white non-pedigree, short-haired, sexually mature rats of both sexes weighing 180–220 g, housed in a certified animal facility.

The experiment was carried out in accordance with developed standard operating procedures. Rats were randomly divided into three main groups according to implant type (xenoperitoneum ECM, UltraPro mesh endoprosthesis, and preserved dura mater). Within each implant group, animals were further subdivided into subgroups based on the bacterial agent (MRSA or E. coli), with six animals per subgroup for each follow-up period (10 and 20 days), as detailed in Table 1.

Table 1 Allocation of animals into the groups

Causative agent	Concentration	Xenoperitoneum ECM		Dura mater		Ultrapro	
		10 days	20 days	10 days	20 days	10 days	20 days
MRSA	109 CFU	6	6	6	6	6	6
E. coli GFP ATCC® 25922GFP™	109 CFU	6	6	6	6	6	6
NaCl (control)	0,9%	6					

The NaCl control group (n = 6) was not stratified by follow-up duration and was used as a baseline reference.

The NaCl control group consisted of six animals and was not subdivided by follow-up period, as it served to establish baseline microbiological and systemic inflammatory parameters in the absence of bacterial contamination.

The absence of external signs of disease and homogeneity of groups by body weight (±10%) were considered criteria for acceptable randomization. Each animal was identified by an individual number applied with a dye to the dorsal surface. Care and housing followed the standards of the Guide for the Care and Use of Laboratory Animals, 8th edition, ILAR, National Academy Press, 2012. All routine animal management procedures were performed in accordance with standard operating procedures.

Implantation procedure and bacterial contamination

Implantation of xenoperitoneum ECM, preserved dura mater, and UltraPro mesh endoprosthesis was performed by suturing the implant into the muscle mass of the anterolateral abdominal wall

After ether anesthesia in an induction chamber (3 L volume, 3 ml diethyl ether, exposure 5 minutes), a standardized defect of the anterior abdominal wall was created on the anterolateral surface of each rat, without entering the free abdominal cavity. The defect (1.5 × 2.5 cm) was closed with the assigned implant, which was sutured "edge to edge" using an atraumatic needle and 4/0 silk sutures. Thereafter, infectious agents were applied to the area of implantation:

1. 200 µl of 10^9 CFU MRSA culture as a classic pathogen of soft tissue infection;
2. 200 µl of 10^9 CFU E. coli GFP ATCC® 25922GFP™ – a clone of ATCC® 25922 containing a multicopy vector encoding green fluorescent protein (GFPmut3), providing fluorescent labeling of gram-negative bacteria.

The bacterial inoculum of 10^9 CFU was intentionally selected to model a worst-case contamination scenario corresponding to severe surgical site infection and to ensure reproducible infection in vivo. Such high-load models are commonly applied in experimental studies evaluating implant performance under conditions of pronounced bacterial challenge.

Contamination was applied according to the study design in the respective subgroups.

Postoperative monitoring

In the postoperative period, the general condition of the animals was assessed daily, including behavioral features, motor activity, response to sound and light stimuli, pelage and skin condition, mucous membrane color, food and water intake, and body weight dynamics. Local status was evaluated by the course of the wound process (hyperemia, local edema, purulent discharge), condition of sutures, and wound healing rate. According to the study design, animals were sacrificed on days 10 and 20 after surgery. Postoperative analgesia was administered using ketoprofen at a dose of 2 mg/kg intramuscularly once daily for 2 days following surgery to minimize postoperative pain and distress.

Blood sampling and microbiological assessment

At the end of the experiment, prior to euthanasia, blood was collected from all experimental animals (n = 72) by intracardiac puncture. Under ether anesthesia, the rat was fixed on the operating table, thoracotomy was performed by sharp dissection, and the apex of the heart was visualized. A needle was introduced into the projection of the left ventricle parallel to the chest wall while gently retracting the syringe plunger. Once blood appeared, advancement of the needle was stopped, and 4–5 ml of blood was collected.

After blood sampling and confirmation of death, material was collected for microbiological examination. The implant was aseptically removed and placed in a sterile Petri dish with 1 ml of 0.9% saline. Subsequent inoculation was performed on oxacillin-containing nutrient agar for MRSA and on ampicillin-containing nutrient agar for E. coli GFP ATCC® 25922GFP™. After 24 hours of incubation at 37 °C, quantitative and qualitative assessment of the microflora was performed.

Determination of circulating immune complexes (CICs)

CICs were determined by precipitation of antigen–antibody complexes from serum using polyethylene glycol (PEG-6000; AppliChem, Germany) prepared in 0.1 M borate buffer (pH 8.4), followed by photometric measurement of the optical density of the precipitate [27].

To assess high molecular weight (HMW) CICs, a 3.5% solution of PEG-6000 was used; for medium molecular weight (MMW) CICs, a 5% solution was used; and for low molecular weight (LMW) CICs, a 7% solution was used. Whole blood without anticoagulant was left to clot for 40 minutes and then centrifuged (ELMI CM70M.07) for 2 hours at 1500 rpm at 37 °C. Serum samples were diluted threefold with borate buffer (pH 8.4). Then, 1.8 ml of PEG-6000 solution (3.5%, 5%, or 7%) was added to three test tubes, and 1.8 ml of borate buffer was added to the control tube. Diluted serum (0.2 ml) was added to each tube and incubated for 120 minutes at room temperature. Optical density of the precipitate was measured at 450 nm. The difference in optical density between control and experimental tubes was calculated and multiplied by 1000 to obtain relative units of CIC content per 1.0 ml of serum.

Complete blood count

A portion of blood (1 ml) with anticoagulant was used for complete blood count. Hemoglobin (g/dl), leukocytes (10^9/l), and erythrocytes (10^{12}/l) were measured using a Mindray BC-3200 hematology analyzer. Differential leukocyte counts (lymphocytes, neutrophils, eosinophils, monocytes, basophils) were determined by standard microscopic examination of smears stained by the Romanowsky–Giemsa method. Hematological parameters obtained from untreated animals were used as reference values.

Statistical analysis. For quantitative data, results are presented as medians and interquartile ranges (Q1–Q3). Nonparametric statistical methods were applied due to the non-normal distribution of the data. Intergroup comparisons were performed using the Mann–Whitney U test for independent samples, while within-group comparisons were assessed using the Kruskal–Wallis test. Qualitative data were analyzed using Fisher's exact test due to the small subgroup size (n = 6). Correlation analysis was performed using Kendall's correlation coefficient (τb). Statistical analysis was conducted using Statistica 12.0 and Microsoft Excel 2019.

Results

General condition of experimental animals

No mortality was observed during the postoperative follow-up period. The general condition of animals in the experimental group (xenoperitoneum ECM) and in the comparison groups (UltraPro mesh endoprosthesis and preserved dura mater) did not differ significantly. Appetite and water intake in the experimental group were comparable to those in the comparison groups. The intensity and nature of motor activity, coordination, and skeletal muscle tone remained normal. Behavioral responses, including reactions to tactile, painful, auditory, and light stimuli, were unchanged. No pathological changes in hair coat, skin, or mucous membranes were observed. No negative dynamics in body weight were detected during the experiment. Thus, the selected inoculum of 10^9 CFU of pathogenic microorganisms did not adversely affect the general condition of the animals in either the control or comparison groups.

Macroscopic evaluation of the implantation area. In the experimental group with xenoperitoneum ECM and MRSA contamination (n = 12), a fully developed abscess was noted in 2 cases (16.7%) (Figure 1).

In the comparison group with UltraPro mesh endoprosthesis, abscess formation after MRSA contamination (n = 12) was also recorded in 2 cases (16.7%). In the group with preserved dura mater, abscess formation was observed in 3 cases (25%) (Table 2).

No macroscopic signs of abscess formation were identified in any group after E. coli inoculation.

Analysis of macroscopic data showed that under bacterial contamination, the postimplantation course in the extracellular bovine-derived peritoneum matrix group was comparable to that in the comparison groups. No statistically significant differences in the incidence of wound complications were detected ($p > 0.05$).

Microbiological assessment of the implantation area

Comparative microbiological assessment was performed on postoperative days 10 and 20 in the implantation zones of xenoperitoneum ECM, UltraPro mesh endoprosthesis, and preserved dura mater after MRSA and E. coli contamination.

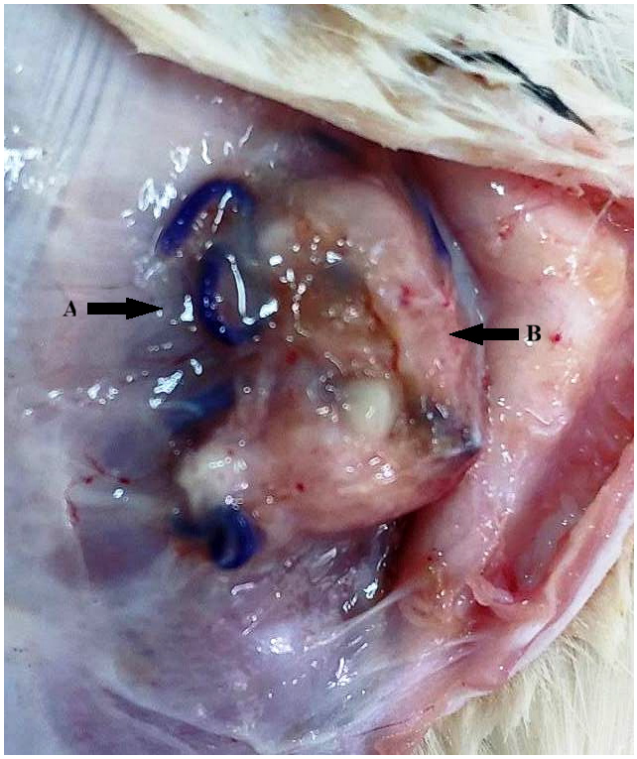

Figure 1 – Defect closure with extracellular bovine-derived peritoneum matrix followed by MRSA infection. Abscess formation on postoperative day 10: A - Implant; B - formed abscess

Table 2 Abscess formation in the implantation zone after MRSA infection

Observation period	Xenoperitoneum ECM	UltraPro mesh endoprosthesis	Preserved dura mater
10 days	1	1	2
20 days	1	1	1
Total, n (abscess / %)	2 / 16.7%	2 / 16.7%	3 / 25%

Statistical analysis: Fisher's exact test.

After E. coli contamination of the implantation zone, no microbial growth was detected on the implants (Figure 2).

In contrast, after MRSA contamination, statistically significant findings ($p < 0.05$) were obtained from wound swabs

Figure 2 – Escherichia coli GFP ATCC® 25922GFP ™ on a transilluminator: A, B, C – experimental groups, no growth; D – control cell (shiny colonies on Tryptic Soy Agar plate)

on postoperative day 10: MRSA was isolated in 60% of all cases (n = 36) (Figure 3). By day 20, no culture growth was observed in the majority of samples, and a significant decrease in pathogen concentration in the wound was recorded (Figure 4).

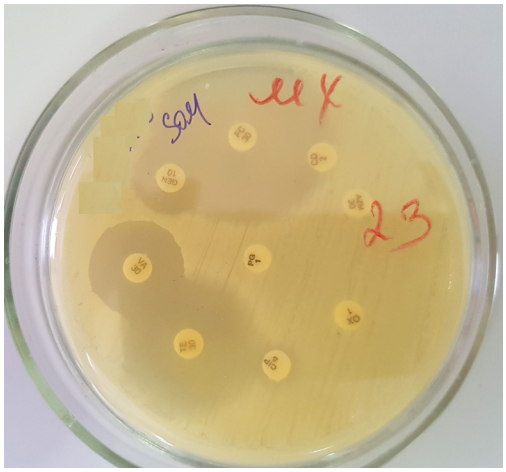

Figure 3 – Growth of MRSA ATCC® 43300 on Mueller–Hinton agar after incubation at 37 °C for 24 h

Intergroup comparative analysis demonstrated that, regardless of the implant used, no statistically significant differences in MRSA concentration were observed on days 10 and 20 ($p > 0.05$) (Figure 5). However, MRSA inoculation in the UltraPro group was highest, reaching 66% on day 10 with a maximal titer of 10^8 CFU.

Kendall's correlation analysis revealed a moderate inverse correlation between the number of days of the experiment and the proportion of positive MRSA cultures from the wound ($\tau b = -0.32$, $n = 72$, $p < 0.05$), indicating that the longer the observation period, the fewer positive cultures were obtained, possibly reflecting the features of the immune response in experimental animals [28]. A similar inverse correlation was observed between the number of days and the rate of MRSA recovery from the implants ($\tau b = -0.48$, $n = 72$, $p < 0.05$).

The titer of microorganisms, whether recovered from the wound or from the implant, also showed an inverse relationship with time: as the number of days increased, the titer decreased ($\tau b = -0.44$, $n = 72$, $p < 0.05$), from 10^8 to 10^3 CFU. By day 20, microbial contamination was either 10^3 CFU or absent.

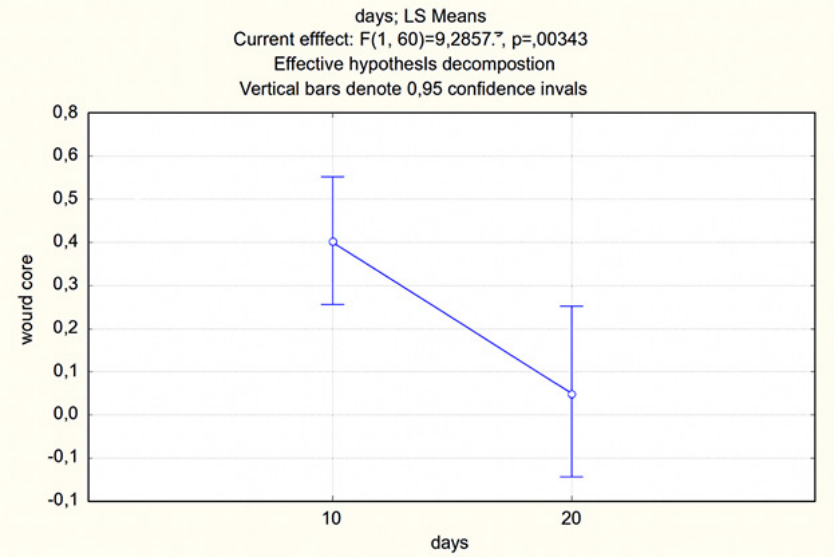

Figure 4 – Dynamics of MRSA recovery from the wound on postoperative days 10 and 20

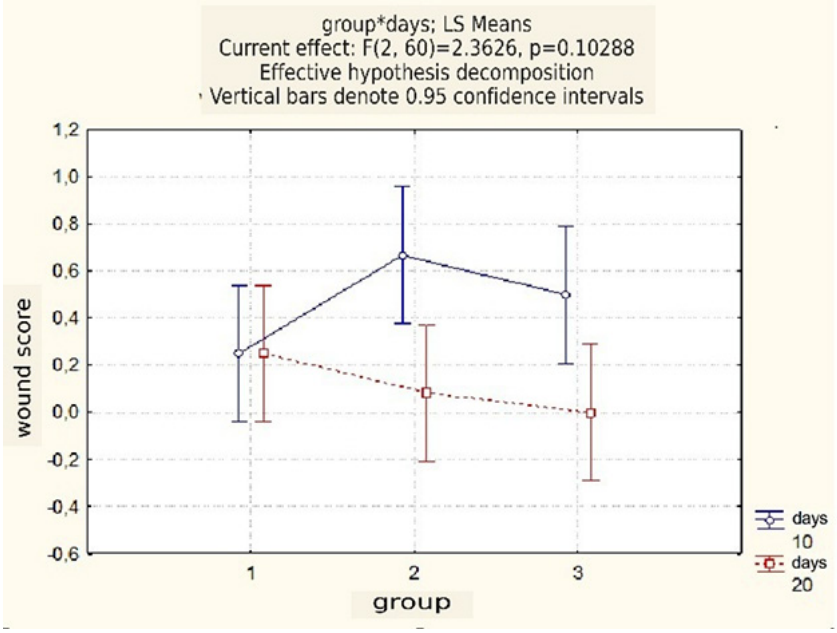

Figure 5 – MRSA recovery from the wound depending on postoperative day and implant type: 1 – xenoperitoneum ECM; 2 – UltraPro mesh endoprosthesis; 3 – preserved dura mater

Circulating immune complexes

The results of CIC measurements in serum of experimental animals after implantation of xenoperitoneum ECM, UltraPro endoprosthesis, and preserved dura mater are presented in Table 3.

Table 3 Circulating immune complexes in serum of experimental animals (IU (Q1-Q3))

Material	Pathogen	Duration, days	HMW c.u.	AMW c.u.	LMW c.u.
ECM	E.coli	10	18.5 (16.5-22.75)	45.5 (37.5-51)	106.5 (94.25-121)
		20	16.0 (14.25-17.75)	37.5 (32.5-40.75)	88 (72.5-95.5)
	MRSA	10	22 (18-34.5)	56 (47-71)	111 (101.5-145.5)
		20	19 15-20.75)	51 (47-55)	64,5 (57.5-82.5)*
UltraPro	E.coli	10	25,2 (18.5-34.75)	53,5 (44-70.5)	118 (107.7-147.5)
		20	27 (17.25-50.25)	62 (40-72.5)	78 (61,25-96.25)*
	MRSA	10	15,5 (13.25-24.5)	40 (30.75-51.5)	111 (97.75-124.5)
		20	16 (12.5-25.5)	31 (15.25-50.5)	49.5 (24.5-86.5)*
DM	E.coli	10	21 (14.5-32)	31,5 (15.25-48.2)	90.5 (72-109.75)
		20	18.5 (13.5-21.25)	28.5 (17.5-32)	68.5 (65.75-75)*
	MRSA	10	*56 (30.5-71)*	*99,5 (62.55-100.75)*	*168 (129-180)*
		20	24 (19.75-43.25)*	43.5 (34-59.75)	90 (83.5-112.35)*
Control	-	-	6.0 (4.0-9.08)	8.25 (5.0-15.4)	12.25 (8.5-21.54)

Asterisks indicate statistical significance (p ≤ 0.05) compared with the 10-day group. Italicized values denote statistical significance between the corresponding E. coli and MRSA groups.
Data are presented as median (Q1–Q3). Statistical analysis: Mann–Whitney U test (between groups), Kruskal–Wallis test (within groups).

CICs reflect the inflammatory process, integrating both antigen accumulation and antibody production, and serve as a general indicator of antigen–immune system interaction. All three CIC fractions increased sharply and significantly by day 10 of the experiment. HMW CICs increased 3.1–4.2-fold, with the maximum elevation observed in the DM group under MRSA contamination (9.3-fold versus control, p = 0.04; p = 0.02 compared with other groups). In the xenoperitoneum ECM and UltraPro groups, no statistically significant differences were found according to implant type or pathogen.

Changes in MMW and LMW CICs were similar: the most marked increase was observed in the DM group under MRSA contamination. In the remaining groups, statistically significant changes relative to controls were recorded, but no significant intergroup differences were found according to pathogen or implant type. By day 20, HMW and MMW CIC levels did not differ significantly from those at day 10, whereas LMW CIC levels decreased in all groups.

The more pronounced decline in low molecular weight (LMW) circulating immune complexes by day 20 in MRSA-infected groups may reflect a faster transition from the acute inflammatory phase to immune complex clearance following an initially stronger antigenic stimulus. MRSA infection induced a more intense early inflammatory response, which was accompanied by higher CIC formation at earlier time points; subsequent reduction of bacterial burden and resolution of acute inflammation may therefore result in a more marked decrease in LMW CIC levels compared with groups infected with E. coli.

Considering the microbiological findings, the more prominent CIC response to E. coli cannot be unambiguously interpreted as an indicator of more severe infection; rather, it may reflect differences in host responses to gram-negative versus gram-positive bacteria. Lipopolysaccharide of gram-negative bacteria is a potent activator of innate immunity and nonspecific defense systems. Comparative analysis showed that preserved dura mater, when infected with both MRSA and E. coli, was associated with a more pronounced systemic inflammatory response. The extracellular bovine-derived peritoneum matrix and UltraPro mesh endoprosthesis produced comparable, and relatively less intense, inflammatory responses.

Complete blood count

Complete blood count data for the studied groups are presented in Table 4.

By postoperative day 7, mild leukocytosis was observed in both experimental and comparison groups ($p \leq 0.05$). Hemoglobin levels decreased slightly (without statistically significant differences). At later time points, no significant differences between groups were recorded.

A comparative evaluation of the systemic response in recipient animals demonstrated that similar hematological patterns and leukocyte differentials were observed in all three implant groups. The maximal leukocyte response was recorded in the DM group under MRSA infection (p = 0.03); no statistically significant differences in leukocytosis between pathogens or implant materials were found in the remaining groups.

The leukocyte response was moderate; a left shift due to band neutrophils was not pronounced. However, an increase in segmented neutrophils was noted in most groups, with the maximum neutrophil response to MRSA observed in all three groups and particularly in the DM group, consistent with the macroscopic findings of abscess formation under MRSA contamination. During the inflammatory phase, leukocyte counts subsequently decreased, especially in groups infected with E. coli (in line with the absence of clinically visible changes in the infection zone). In groups with MRSA, no significant progressive increase in leukocytosis or neutrophil count was observed. By day 7 of the experiment, leukocyte counts approached control values in all studied groups.

Thus, experimental animals with bacterial contamination of xenoperitoneum ECM and comparison materials demonstrated an adequate response to infectious challenge, characterized by a localized inflammatory zone and leukocytosis, without signs of systemic purulent–septic complications.

Table 4 Complete blood count with differential in the studied groups

Material	Pathogen	Duration, days	Leucocytes 109/l	Rod nuclear cells%	Segmento-nuclear neutrophils %	Eosinophils%	Lymphocytes %
ECM	E.coli	10	7.35 (4.85-8.27)	1 (0-3.5)	*18* (16-21.5)	2 (2-3.5)	78 (75-81)
		20	5.15 (3.95-6.27)	6 (3-6)	10 (8-12)	5 (4-7.5)	83 (74-86)
	MRSA	10	8.35 (7.6-12.2)	4 (2.5-4)	14.5 (9.5-18.8)	1 (0-3.5)	81.5 (73-85)
		20	7.65 (6.0-7.8)	5 (1-6)	11 (8-14.8)*	2 (1.3-5)*	89 (83-94)
UltraPro	E.coli	10	7.35 (6.9-10.55)	3 (2-7.5)	7 (5.3-8)*	1,0 (0.3-1.8)*	89 (89-89.8)
		20	6.95 (4.35-8.95)	2.5 (2-3.8)*	10 (7-10)*	4.5 (0.5-7.8)*	85 (81-86)
	MRSA	10	10.9 (9.12-17.25)	0	20 (10-27)	0	80 (73-90)
		20	6.7 (5.42-5.4)	3 (2-4.8)	11.5 (5.3-18.5)	3.5 (0.75-4)	84 (73-92.8)
DM	E.coli	10	7.7 (6.05-11.22)	1 (0-1)	17 (10-22)	0 (0-2)	81 (56-84)
		20	4.4 (4.1-4.9)	1 (0,3-1)	14.5 (13-18)	0 (0-0.8)	85.5 (79-88.3)
	MRSA	10	*18.25* (6.0-22.3)	1 (0.5-1.5)	*23* (22-35.5)	1 (0.5-2.0)	76 (62-77)
		20	7.35 (5.47-7.8)	1 (0.3-3.3)	15.5 (12.5-19)	0.5 (0-1.8)	83 (82-84.8)
Control	-	-	4.85 (4.0-6.9)	1.0 (0-2.0)	11 (5.0-16.8)	3.6 (1-2.3)	79 (75-86)

An asterisk indicates statistical significance ($p \leq 0.05$) compared with the 10-day group. Italicized values denote statistical significance between the corresponding E. coli and MRSA groups.
Data are presented as median (Q1–Q3). Statistical analysis: Mann–Whitney U test.

Discussion

The early postoperative period after experimental prosthetic repair under high bacterial contamination is accompanied by a pronounced inflammatory response. This observation is consistent with clinical data indicating a high incidence of infectious complications in emergency surgery [6].

In the present experiment, bacterial contamination of the implantation zone was modeled using the most typical pathogens for anterior abdominal wall surgery: a multiresistant Staphylococcus aureus strain (MRSA) and E. coli. Analysis of the obtained data showed that the inflammatory response in the implantation zone was more severe under MRSA infection, with statistically significant differences compared with groups contaminated by E. coli. However, the comparison materials—UltraPro synthetic endoprosthesis and preserved dura mater—did not demonstrate any clear advantages over the tested xenoperitoneum ECM. Microbiologically, no statistically significant differences in bacterial growth were observed between the various implants.

The study of CICs in serum, including in vitro testing, demonstrated that MRSA contamination of the implantation zone led to a rapid increase in CIC levels, mainly due to the HMW fraction (DM group), which exceeded control values by ninefold. CICs are an integral marker with low specificity: inflammation, wound healing, and scarring are typically accompanied by their formation. The acute inflammatory process, which macroscopically manifested as abscess formation, was likely a trigger for CIC accumulation in the studied groups. In the xenoperitoneum ECM group, CICs of all classes also increased, but less markedly than in the DM comparison group.

The more pronounced CIC response to E. coli cannot be regarded solely as a marker of infection severity; rather, it likely reflects the differential host response to gram-negative and gram-positive agents. As mentioned, lipopolysaccharide of gram-negative bacteria is a strong stimulator of innate and nonspecific immunity. Comparative analysis showed that preserved dura mater, when infected with both MRSA and E. coli, was associated with a more severe systemic inflammatory response. In contrast, the xenoperitoneum ECM and UltraPro mesh endoprosthesis elicited a relatively lower inflammatory reaction.

The disproportionately pronounced inflammatory response observed with preserved dura mater may be related to its structural and biological characteristics. Unlike decellularized ECM scaffolds, preserved dura mater may retain residual antigenic components and undergo limited remodeling, which can prolong inflammatory signaling. In addition, its dense collagen structure and lower porosity may impair early cellular infiltration and neovascularization, thereby delaying integration and promoting sustained inflammatory reactions, particularly under conditions of bacterial contamination.

Overall, hematological and microbiological data confirm that the extracellular bovine-derived peritoneum matrix behaves comparably to a modern composite synthetic mesh (UltraPro) and better than preserved dura mater in terms of systemic inflammatory burden, while ensuring adequate local inflammatory response and infection control.

From a clinical perspective, the findings of this study suggest that xenoperitoneum ECM may represent a viable alternative to synthetic meshes in contaminated or potentially

contaminated surgical fields. The comparable microbiological outcomes and lower systemic inflammatory burden observed with this biologic scaffold support its potential use in complex ventral hernia repair, emergency surgery, and high-risk patients where the risk of infection is increased. These results provide an experimental rationale for further clinical evaluation of peritoneum-derived ECM in abdominal wall reconstruction under compromised conditions.

This study has several limitations. First, the follow-up period was limited to 20 days and does not allow assessment of long-term outcomes. Second, the experimental model was based on rodents, which may not fully reproduce the complexity of human abdominal wall reconstruction. Third, biomechanical properties of the repaired abdominal wall and collagen remodeling were not evaluated and should be addressed in future studies.

Conclusion

In this study, the extracellular bovine-derived peritoneum matrix (xenoperitoneum ECM) was evaluated for the first time under conditions of experimental bacterial contamination [29].

Based on the obtained data, experimental animals subjected to bacterial contamination of xenoperitoneum ECM and comparison materials (UltraPro and preserved dura mater) demonstrated an adequate response to the infectious challenge, characterized by a limited inflammatory zone and leukocytosis without signs of systemic purulent–septic complications.

The findings of the present experimental study support further research, including clinical trials, on the use of xenoperitoneum ECM for the repair of anterior abdominal wall defects in infected and conditionally infected settings.

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Comprehensive Gene Expression Analysis of Intraductal Papillary Mucinous Neoplasms (IPMN) in Pancreatic Cancer: Insights into Biomarkers and Therapeutic Targets

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ABSTRACT

Introduction: Pancreatic cancer remains a highly deadly malignancy, with its progression driven by genetic changes and microenvironmental influences. Early lesions like intraductal papillary mucinous neoplasms (IPMN) offer valuable insights into precursor biology and potential biomarkers for early detection. This research focused on examining gene expression differences across pancreatic IPMN subtypes, IPMA (adenoma), IPMC (carcinoma) and mixed IPMN, by comparing them to normal pancreatic tissues through analysing publicly available microarray data (GSE19650). The main goal was to identify differentially expressed genes and enriched biological pathways, which could shed light on the mechanisms underlying pancreatic tumour development.

Methods: This retrospective bioinformatics study examined gene expression data from the GEO database (GSE19650), including normal pancreatic tissues and IPMN subtypes. Differential gene expression was determined through standard pre-processing, normalization and statistical testing employing adjusted p-value thresholds to identify significant genes. Functional enrichment analyses, such as Gene Ontology and pathway analysis were conducted to explore biological relevance. The study did not involve any unpublished data or patient-identifiable information. When clinical data were available, survival analysis was performed. All statistical analyses were conducted using R software (version 4.x) with packages like limma and clusterprofiler applying appropriate cut-off thresholds and multiple testing corrections.

Results: Differential expression analysis identified unique molecular signatures among IPMA, IPMC and mixed IPMN groups. Genes like S100P showed increased expression in neoplastic tissues, whereas genes such as CELP and CELA2B were downregulated indicating a loss of normal pancreatic function. Enrichment analyses identified pathways involved in nuclear division and extracellular matrix organisation. However, survival analysis did not find statistically significant correlations.

Discussion: These findings underscore molecular differences among IPMN subtypes, confirming their biological diversity. The increased expression of immune-modulatory and tumour-related genes supports their known roles in pancreatic cancer development, while the reduced expression of digestive enzyme genes indicates a disruption in normal tissue function. Although survival analysis showed limited associations, this may be due to the small dataset and few clinical variables. Overall, the study emphasises the significance of pathway-

level changes such as proliferative signalling and extracellular matrix remodelling, which may drive neoplastic transformation and progression.

Conclusions: This study identifies distinct gene expression patterns and enriched pathways across IPMN subtypes, offering insights into early pancreatic tumour development. Although biomarker candidates like S100P show promise, limited survival correlations highlight the complexity of prognostication. Larger datasets with comprehensive clinical information are required to refine molecular markers and advance personalised therapeutic approaches in pancreatic cancer research.

Keywords: Pancreatic cancer, Gene expression profiling, Intraductal papillary mucinous neoplasms (IPMN), Biomarker.

Introduction

Cancer is a broad term for a group of diseases characterised by the uncontrolled growth and division of abnormal cells in the body. They result from a complex interplay of genetic changes, including mutations in tumour suppressor genes, DNA repair genes, and proto-oncogenes [1]. Pancreatic cancer is a deadly condition caused by a combination of genetic and environmental factors. Research indicates that individuals with a family history of pancreatic cancer are more likely to develop adenocarcinoma, suggesting a significant genetic susceptibility [2]. Epidemiological studies have shown that the incidence of pancreatic cancer varies globally, with higher rates in North America, Western Europe, Australia, and New Zealand [3]. The most common site of pancreatic cancer is the head of the pancreas. Approximately 60-70% of cases occur in this region. This is clinically important because tumours in the pancreatic head can lead to early jaundice, a common symptom that may enable earlier diagnosis [4]. Understanding the genes involved in tumour formation can provide valuable insights into the disease process and help guide treatment strategies. The exocrine pancreas exhibits a higher mutation frequency than the endocrine pancreas. [5,6].

Compared to the general population, genetic disorders such as BRCA1 and BRCA2 are associated with higher relevant risks for pancreatic cancer [7]. Key genes involved in pancreatic cancer include KRAS, CDKN2A, TP53, and SMAD4. Mutations in KRAS are responsible for around 90% of cases [7]. Axon guidance pathway genes are among several genetic abnormalities observed in pancreatic carcinogenesis. A recent study identified unique gene expression profiles supporting the pancreatic duct as the origin of the cancer [8]. The hereditary nature of pancreatic cancer is linked to genetic disorders, including BRCA and PALB2 mutations. The CDKN2A gene encodes the p16INK4a protein, which is often inactivated in pancreatic cancer through mutation and deletion. This disrupts cell cycle regulation, permitting abnormal cellular division. Similarly, the SMAD4 gene, involved in TGF-Beta signalling pathways, is mutated in 55% of pancreatic adenocarcinomas (PAAD). Loss of SMAD4 functions leads to increased cell proliferation and resistance to apoptosis [9,10]. Mutations in inherited genes such as BRCA2, ATM3, PALB2, BRCA15, STK116, CDKN2A7, and mismatch repair genes are associated with an increased risk of pancreatic cancer. Additionally, new pancreatic risk loci including 17q25.1, 7p13, and 3q29 have been identified [11].

Gene expression analysis in pancreatic cancer has become a vital tool for understanding the disease's molecular mechanisms and identifying potential therapeutic targets. Recent advances in high-throughput sequencing technologies have enabled researchers to define the genetic alterations associated with pancreatic cancer [12]. A recent study demonstrates the potential use of gene expression profiles as predictive biomarkers for patient classification and personalised therapy. The study identified a subtype of pancreatic cancer characterised by the activation of the Hedgehog signalling pathway, suggesting that targeting this pathway could be a promising therapeutic approach for patients with this subtype. A gene expression study was carried out using micro gel-embedded pancreatic cancer spheroids. Their work provides a platform for real-time PCR to measure gene expression variations, allowing high-throughput screening and supporting the design of patient-specific treatments. The Weighted Gene Co-expression Network Analysis (WGCNA) was employed to identify core modules linked to pancreatic cancer types, revealing significant ncRNA and transcription factors (TFs) that regulate core module genes. This can be applied to integrating gene expression data for identifying different cancer types and offers a platform for future research in precision medicine [13].

Despite significant progress in pancreatic cancer genomics, most current studies mainly focus on pancreatic ductal adenocarcinoma (PDAC) as a single entity or rely on limited subtype-specific analyses [14,15]. In contrast, this study offers a comparative and integrative transcriptomic analysis across different stages of intraductal papillary mucinous neoplasms (IPMA, IPMC, and IPMN) using publicly available high-throughput datasets. By systematically identifying differentially expressed genes, constructing protein–protein interaction networks, and combining expression validation with survival analyses across multiple platforms, it provides stage-specific molecular insights into IPMN progression towards pancreatic cancer. The study uniquely highlights the combined interpretation of upregulated oncogenic markers and downregulated acinar cell–associated genes, thereby enhancing the understanding of early tumourigenic events, loss of pancreatic differentiation, and potential biomarker candidates. These findings expand upon existing research by linking transcriptomic alterations to functional pathways and interaction networks, offering a more comprehensive framework for biomarker discovery and therapeutic target identification in pancreatic neoplasms.

Methods

Data collection

The raw data for this investigation was sourced from the Gene Expression Omnibus (GEO) database [16]. Specifically, expression profile array data from clinical samples were downloaded under the accession ID GSE19650, focusing on pancreatic cancer. This dataset contains samples of normal and malignant epithelial cells from frozen tissue sections. The

samples include normal main pancreatic duct (GSM490138-GSM490144), intraductal papillary-mucinous adenoma (IPMA) (GSM490145-GSM490149), intraductal papillary-mucinous carcinoma (IPMC) (GSM490151-GSM490156) and intraductal papillary-mucinous neoplasm (IPMN) (GSM490157-GSM490159) from 22 patients. The evaluation aimed to identify differentially expressed genes, applying four distinct criteria to differentiate between the various sample types effectively.

Identification of DEGs

In our study, differential gene expression (DEGs) was conducted using GEO2R, a web-based analysis tool provided by the National Centre for Biotechnology Information (NCBI), which enables comparison of gene expression profiles across experimental conditions within Gene Expression Omnibus (GEO) datasets (http://www.ncbi.nlm.nih.gov/geo/geo2r) in pancreatic cancer. The datasets GSE19650 were divided into three distinct groups under standard conditions: intraductal papillary mucinous adenoma (IPMA), intraductal papillary mucinous carcinoma (IPMC) and intrapapillary mucinous neoplasm (IPMN), each compared independently against normal pancreatic tissue samples. The comparisons included IPMA vs. Normal, IPMC vs. Normal, and IPMN vs. Normal. To ensure both biological relevance and statistical precision, strict threshold criteria were applied, including an absolute log2 fold change >1 (at least a two-fold change) and a P-value below 0.05. Differential expression was identified by fitting linear models to gene expression data across the defined comparison groups, followed by statistical testing to find genes with significant expression differences compared to normal pancreatic tissue. DEGs were classified into up-regulated and down-regulated gene groups based on the direction of expression changes compared to normal tissue.

Protein-protein interaction (PPI) Network

The protein-protein interaction (PPI) and STRING database for DEGs identified in IPMA, IPMC and IPMN datasets were performed [17]. The DEGs were mapped to the STRING database to create PPI networks illustrating both direct and indirect connections among proteins involved in pancreatic neoplasia. In these networks, nodes denote individual proteins encoded by the DEGs, while edges represent interactions

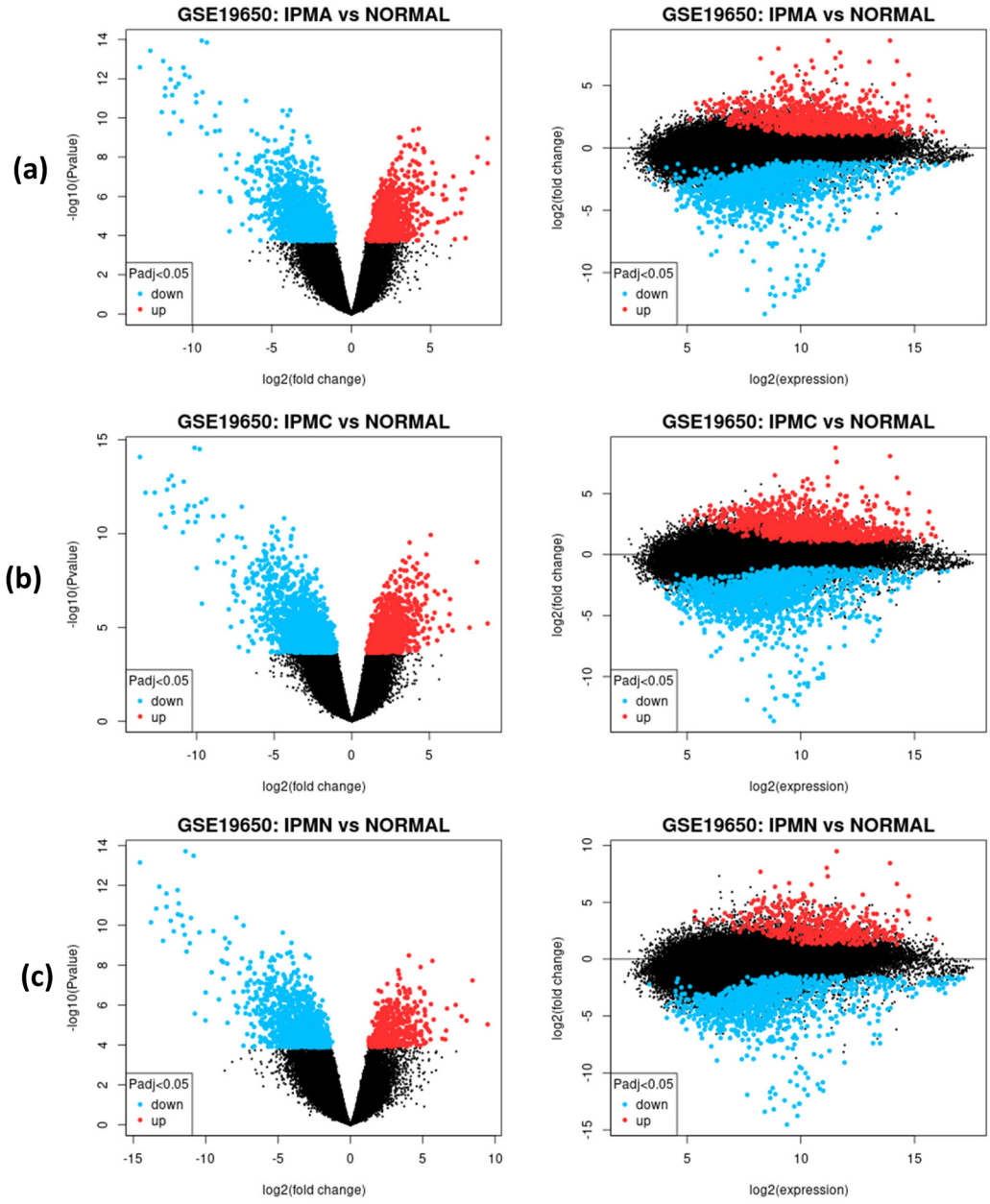

Figure 1 – Differentially expressed genes (DEGs) of (a) IPMA vs Normal, (b) IPMC vs Normal and (c) IPMN vs Normal using volcano and MD plots

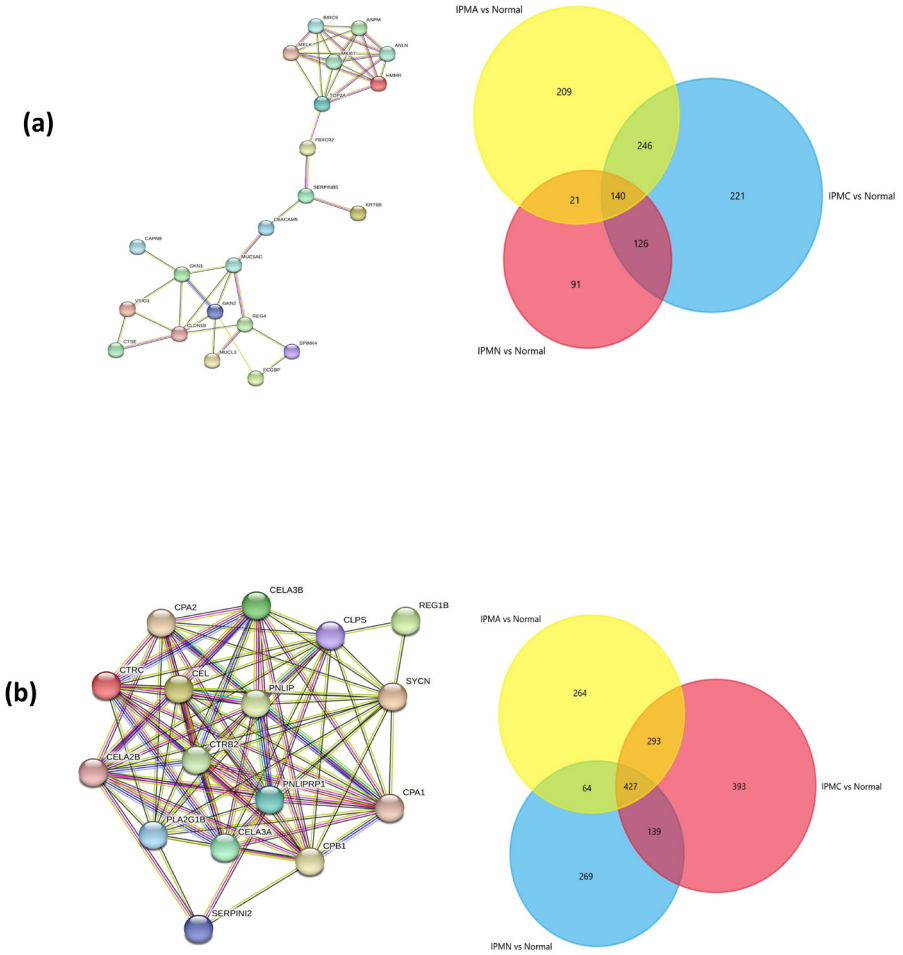

Figure 2 – Protein-protein interactions derived from the STRING database. A Venn diagram analysis was conducted for both (a) upregulated genes and (b) downregulated genes

or relationships between protein pairs. Analysing these PPI networks helps identify hub proteins with high connectivity, which are often crucial regulators of cellular homeostasis and disease progression. This comprehensive approach aids in pinpointing key regulatory proteins and interaction modules that may serve as potential biomarkers or therapeutic targets for pancreatic cancer.

Survival analysis

The GEPIA database (Gene Expression Profiling Interactive Analysis, http://gepia.cancer-pku.cn/index.html) is a freely accessible tool for detecting gene expression profiles in both tumor and normal samples [17]. UALCAN (http://ualcan.path.uab.edu/) is a web tool that provides an in-depth analysis of transcription data using MET500 and TCGA data [18]. These tools, GEPIA and UALCAN, were used to analyze survival rates and gene expression relationships in pancreatic adenocarcinoma (PAAD), focusing on genes common across different cancer stages.

Gene ontology and pathway analysis of DEGs

The differentially expressed genes (DEGs) identified from the IPMA, IPMC and IPMN datasets were analyzed using gene ontology (GO), focusing on genes with a log2 fold change greater than 5. This analysis was conducted separately for upregulated and downregulated genes to understand their functional roles. The GO analysis classified these genes into three primary categories: biological processes, cellular components, and molecular functions. The biological processes category examines the various physiological pathways and activities the genes are involved. The cellular components category identified the specific locations within the cell or extracellular environment where these genes are active. Finally, the molecular functions category detailed the biochemical activities and interactions mediated by these genes. This methodological approach allowed for a comprehensive understanding of the functional consequences of DEGs in the context of pancreatic cancer.

Results

Identification of DEGs

From the dataset (GSE19650) the DEG analysis was conducted by GEO2R using the limma program, with selection criteria of an adjusted p-value of <0.05 and a log2 fold change of >1. Figure 1 depicts the volcano plot and the MD plot for each gene expression profile data. The red and blue spots show genes that are strongly up-regulated or down-regulated, respectively. A total of 1,371 overlapping DEGs, including 512 up-regulated and 859 down-regulated were discovered using three separate criteria: IPMA vs Normal, IPMC bs Normal and IPMN vs Normal. As a result, this strategy made it easier to create volcano plots for each dataset.

Protein-protein interaction (PPI) Network

The protein-protein interaction (PPI) network was performed in the STRING database for the top 20 Upregulated genes from all three criteria as a whole and the same was performed for downregulated genes. A Venn diagram was performed for the three criteria to find the number of common

genes. In the case of upregulation Figure2a shows that 140 genes are common across the three criteria while in the case of downregulation Figure 2b shows that 427 genes are common. The figure also represents the constructed protein-protein interaction (PPI) network and the Venn diagram illustrates the genes shared among the criteria.

Survival analysis

A comparative mRNA expression analysis of the common genes was performed for pancreatic cancer using the GEPIA platform. Both upregulation and downregulation expression studies have been done. Figure 3, The box plots reveal that CLDN18, AFAP1-AS1, S100P, and CTSE are notably

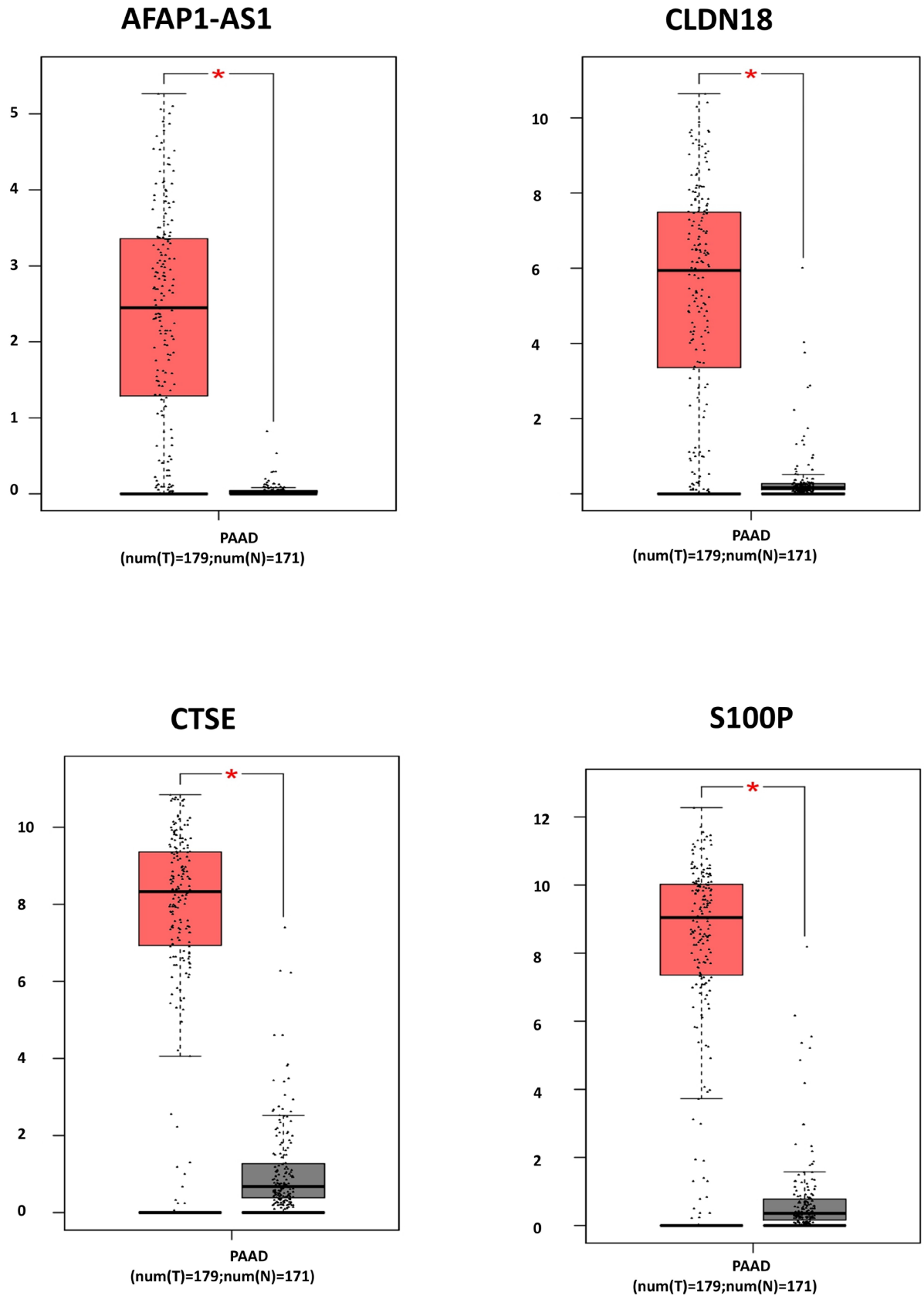

Figure 3 – The Significantly up-regulated expression of AFAP1-AS1, CLDN18, CTSE, and S100P in tumor samples compared to normal samples, with a sample size of 179 for tumor and 171 for normal tissues

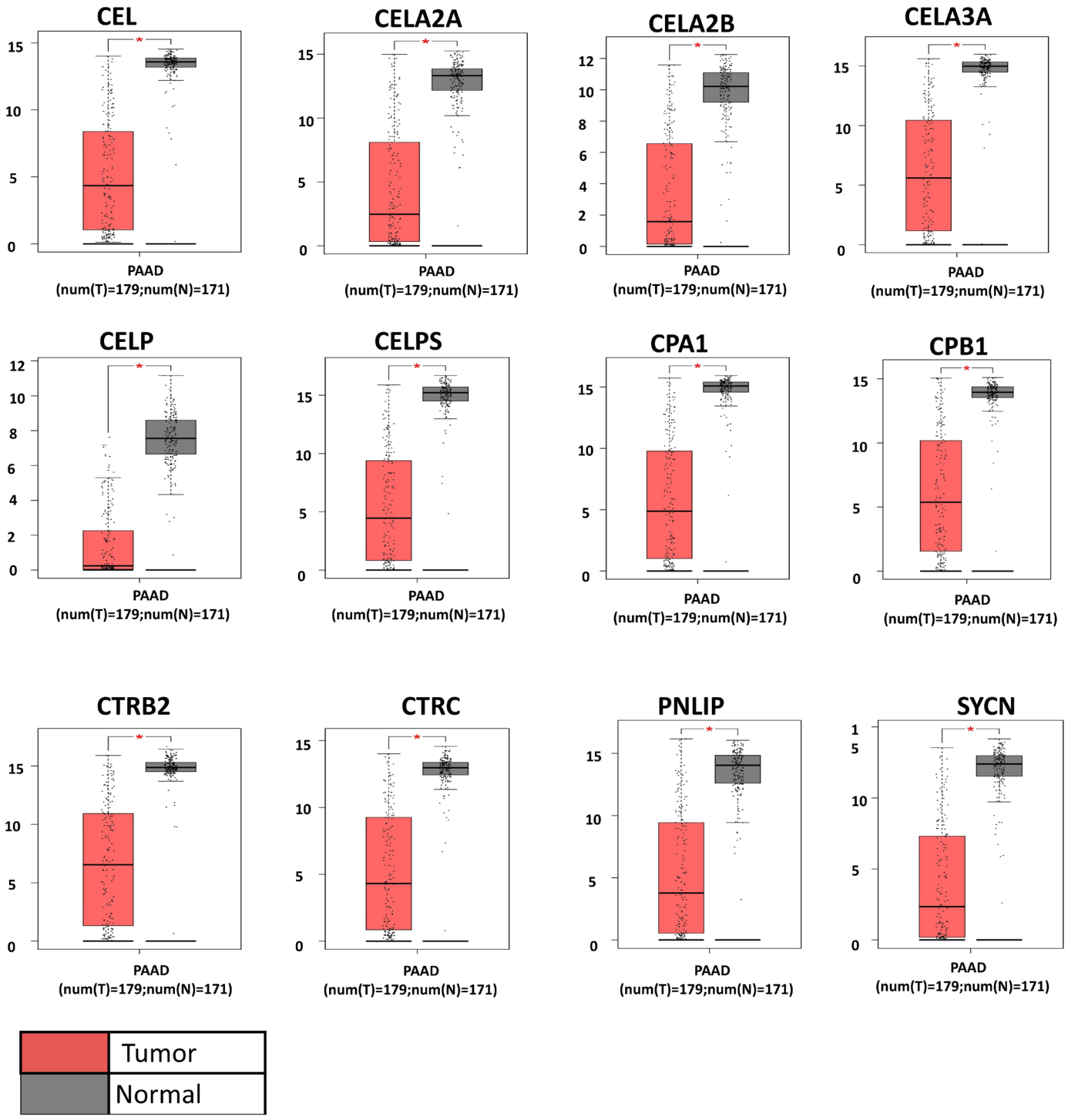

Figure 4 – Significantly down-regulated expression of CEL, CELA2A, CELA2B, CELA3A, CELP, CELO5, CPA1, CPB1, CTRB2, CTRC, PNLIP, and SYCN in tumor samples compared to normal samples, with a sample size of 179 for tumor and 171 for normal tissues

upregulated in pancreatic adenocarcinoma (PAAD) compared to normal pancreatic tissues. Tumor samples show significantly higher median expression levels and broader expression ranges, indicating consistent overexpression among patients. These differentially expressed genes (DEGs) imply that these genes may play roles in tumour initiation, progression and epithelial or invasive activities in pancreatic cancer. Their persistent upregulation highlights their potential as diagnostic biomarkers, although they may not serve as standalone prognostic markers. Figure 4, This figure illustrates the decreased expression of genes such as CEL, CELA2A, CELP, CELA3A, CELA2B, SYCN, CELPS, CPA1, CTRB2, CTRC, PNLIP, and CPB1 in pancreatic adenocarcinoma (PAAD) compared to normal pancreatic tissues. Normal samples show consistently higher levels, indicating preserved pancreatic exocrine and acinar cell functions. Many of these genes encode digestive enzymes, suggesting a loss of pancreatic exocrine activity in cancer. Their collective downregulation underscores dedifferentiation as a key molecular characteristic of pancreatic cancer progression.

The correlation between the expression level of the common gene in individual stages of cancer was performed using the UALCAN platform. For upregulation, there were 4 common genes from IPMA, IPMC and IPMN. The analysis of gene expression in pancreatic adenocarcinoma (PAAD) across different cancer stages, using UALCAN data based on TCGA samples, reveals significant upregulation of AFAP1, CTSE,

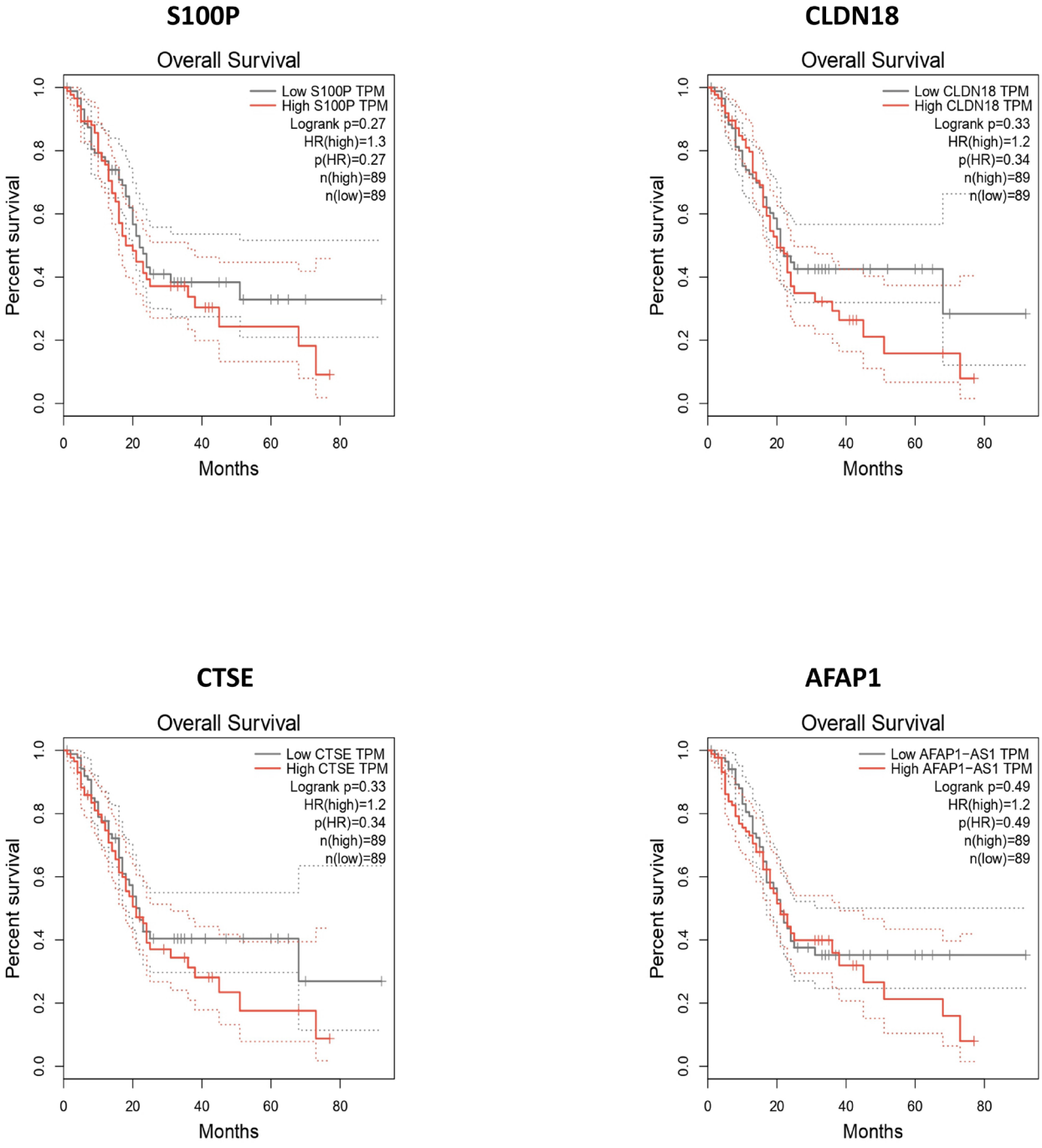

Figure 5 – Overall survival curves of upregulated genes in patients diagnosed with pancreatic cancer, sourced from the TCGA database

CLDN18, and S100P compared to normal pancreatic tissues (Supplementary 1). For downregulation 12 common genes from IPMA, IPMC and IPMN. The analysis of gene expression in pancreatic adenocarcinoma (PAAD) across different cancer stages, using UALCAN data based on TCGA samples, reveals significant downregulation of CEL, CELA2A, CELA2B, CELA3A, CELP, CELO5, CPA1, CPB1, CTRB2, CTRC, PNLIP, and SYCN (Supplementary 2).

The overall survival analysis is also done for both upregulation and downregulation genes. In terms of upregulation, the results indicated that the significantly higher expression of genes had no statistical effect on the patient's overall survival Figure 5. In terms of downregulation, the results indicated that the relatively low expression of genes had no statistical influence on the patient's overall survival Figure 6.

Gene ontology and pathway analysis of DEGs

The gene ontology analysis highlights several biological processes, cellular components and molecular functions that are intricately linked to the prognosis in pancreatic cancer patients. It was performed for the genes that have >3 log2 fold change for upregulation and >5 for downregulation genes (Supplementary 3). shows the enrichment analysis of upregulated genes and (Supplementary 4) shows the enrichment analysis of downregulated genes.

Discussion and Limitations

Pancreatic cancer's complexity arises from a combination of genetic and environmental factors. Geographical variations in incidence rates, with higher rates in Northern America and

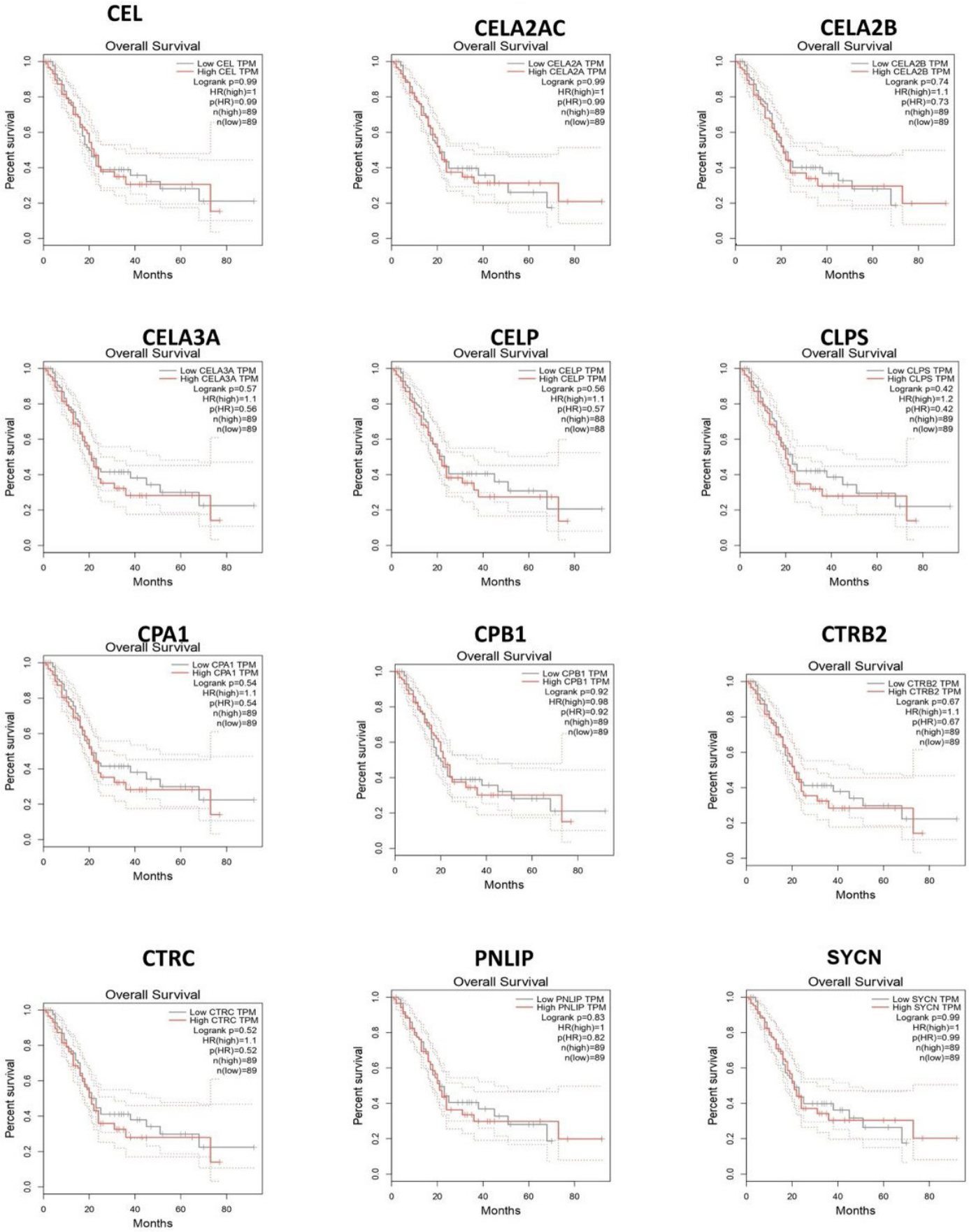

Figure 6 – Overall survival curves of downregulated genes in patients afflicted with pancreatic cancer, obtained from the TCGA database

Western Europe, suggest that lifestyle and environmental factors significantly influence the disease's development. In India, the pancreas ranks 24th with 10860 new cases (1.03%) and 18th in mortality [19]. Tumors most commonly occur in the head of the pancreas, which often leads to early detection due to symptoms like jaundice. Recent advancements in gene expression analysis have significantly improved our understanding of pancreatic cancer [20]. High-throughput sequencing and gene regulatory

network analysis are paving the way for personalized treatment approaches. These technologies help identify potential biomarkers and therapeutic targets, enabling the development of more effective and individualized treatments.

The analysis of gene expression profiles from dataset GSE19650 reveals significant differences between various pancreatic intraductal papillary mucinous neoplasms and normal pancreatic tissues. Three types of samples (IPMA, IPMC, and IPMN) were compared using volcano plots and mean-difference (MD) plots. IPMA shows extensive genetic alterations with both upregulation and downregulation of numerous genes. IPMC exhibits a distinct molecular signature characterized by a notable number of differentially expressed genes (DEGs). IPMN displays a broader distribution of DEGs, indicating complex regulatory changes compared to normal tissues. These findings suggest potential biomarkers for early detection and subtype differentiation of IPMN, as well as novel therapeutic targets for further research and intervention development in pancreatic neoplasms. With the genes, the PPI network is constructed in STRING Database and used for further analysis.

The expression analysis of genes conducted using GEPIA provides a detailed view of both upregulation and downregulation patterns in pancreatic cancer. Among the upregulated genes, S100P stands out with notably higher expression levels compared to the other genes analysed. S100P plays a significant role in the immune microenvironment, and tumorigenesis of pancreatic cancer [21]. This suggests a potential prominent role for S100P in the molecular mechanisms driving pancreatic cancer progression. In contrast, the analysis of downregulated genes highlights CELP and CELA2B as having significantly lower expression levels relative to other downregulated genes [22]. CELP and CELA2B, responsible for encoding pancreatic exocrine enzymes, are significantly downregulated in pancreatic cancer, indicating a loss of acinar cell differentiation and impaired normal digestion as the tumour progresses. Their decreased expression aligns with acinar to ductal metaplasia (ADM), an important early event in pancreatic tumour development. The persistent suppression of these genes across different disease stages suggests they may serve as markers for pancreatic tissue de-differentiation rather than directly influencing prognosis. This emphasises their value in disease characterisation and identifying early pathological transformation rather than predicting patient survival. This substantial decrease in expression indicates that these genes might be crucial in maintaining normal pancreatic function, and their reduced activity could be linked to the development and progression of pancreatic cancer. Furthermore, the relative expression levels of these upregulated and downregulated genes correlate well with their behaviour across different stages of pancreatic cancer compared to the normal stage. This correlation underscores the importance of these genes in the disease's progression and suggests that they could serve as potential biomarkers for different stages of pancreatic cancer.

The overall survival analysis of differentially expressed genes (DEGs) in pancreatic cancer did not show statistically significant differences in survival between groups. This suggests that the expression levels of these genes alone do not significantly influence overall survival outcomes in the studied cohort. While these genes may still play roles in pancreatic cancer biology, their expression levels do not appear to predict patient survival strongly. This lack of statistical significance underscores the complexity of pancreatic cancer, implying that factors beyond gene expression levels-such as genetic variations, environmental factors, and treatment responses-likely have a more significant impact on patient outcomes. It highlights the need for larger studies that integrate diverse molecular and clinical parameters to better understand the disease and improve prognostic models. The main reasons that contribute to the lack of significant survival correlations include sample size limitations, multifactorial prognosis, tumor heterogeneity, data constraints, and treatment variability. Small sample sizes and uneven stage distribution in publicly available datasets like TCGA can decrease statistical power when patients are divided into high- and low-expression groups. Survival outcomes in pancreatic cancer are also heavily influenced by non-transcriptional factors such as surgical respectability, response to chemotherapy, and comorbid conditions which are not fully captured in transcriptomic analyses. Moreover, prognosis is often driven by pathway-level dysregulation and multigene signatures rather than effects from single genes. Overall, these factors highlight the necessity for integrative approaches that combine gene expression data with clinical, genomic and therapeutic information to enhance prognostic accuracy in pancreatic cancer.

The enrichment analysis performed in this study sheds light on the biological pathways and processes that may be implicated in pancreatic cancer progression and patient outcomes. In biological processes, most of the gene count can be seen in nuclear division (meiotic too). Meiotic nuclear division 1 (MND1) overexpression increased cell proliferation, migration, and invasion in vitro. Overexpression of MND1 in pancreatic cancer cells led to increased mRNA and protein levels of MND1, resulting in enhanced cell proliferation, migration, and invasion. This was evidenced by CCK8, wound healing, and transwell assays [23]. In the cellular component, mostly, condensed chromosome centromeric region and collagen-containing extracellular matrix share max no of genes. Chromosome instability, common in pancreatic cancer, arises from errors in chromosome segregation. The kinetochore, which binds to the centromere, plays a crucial role in preventing this instability. Recent findings indicate that the alpha-satellite DNA at centromeres is actively transcribed and tightly regulated, suggesting RNA's potential role in maintaining chromosome stability [24]. Pancreatic ductal adenocarcinoma is marked by a dismal prognosis, largely due to its dense fibrotic response rich in collagen-containing extracellular matrix (ECM). This collagen-rich environment creates a supportive niche for cancer stem cells (CSCs) [25]. In the Molecular functions, microtubule binding and extracellular structural constituent share max no of genes. In pancreatic cancer, microtubule binding and extracellular structural constituents play pivotal roles influenced by shared genetic underpinnings. Microtubule-binding proteins are critical for cell division and intracellular transport, processes vital for cancer cell proliferation and metastasis. Meanwhile, components of the extracellular matrix (ECM) provide structural support and signalling cues that promote tumor growth and invasion. Understanding these molecular functions helps elucidate how dysregulation in pancreatic cancer can lead to aggressive tumor behaviour and poor patient outcomes, highlighting potential targets for therapeutic intervention aimed at disrupting these processes [26, 27].

While this study offers valuable insights into the molecular landscape of pancreatic cancer and IPMN progression, certain limitations should be recognised. First, the analysis is based on publicly available transcriptomic datasets, which may be limited by small sample sizes, diverse patient populations, and incomplete clinical annotations. Second, gene expression–based findings were not supported by experimental validation

at the protein or functional level, which is necessary to confirm biological relevance. Third, survival analyses relied on single-gene expression stratification, which may not fully capture the prognostic complexity of pancreatic cancer driven by pathway-level dysregulation and multigene interactions. Additionally, important clinical factors such as treatment regimens, tumor microenvironment composition, and patient comorbidities were not incorporated into the analysis. Future studies incorporating larger cohorts, multi-omics data and experimental validation will be essential to enhance the translational relevance of these findings and support their application in precision oncology.

Conclusion

In conclusion, this comprehensive transcriptomic analysis of the GSE19650 dataset reveals the molecular changes involved in the progression of pancreatic intraductal papillary mucinous neoplasms towards invasive pancreatic cancer. Differential expression of genes such as S100P, CELP, and CELA2B highlights their role in disrupted epithelial differentiation, extracellular remodelling, and oncogenic signaling during IPMN progression, supporting their potential as stage-specific diagnostic and prognostic biomarkers. Although survival analyses did not find statistically significant links between each gene's expression and clinical results, this probably highlights the complex multifactorial process of pancreatic tumour development, where gene expression interacts with tumor microenvironment, genomic instability and varied treatments. Functional enrichment analyses further highlight key biological processes, such as nuclear division–related pathways involving MND1, indicating a potential role in abnormal cell cycle control and genomic stability that could contribute to malignant transformation. Moreover, the enrichment of extracellular matrix (ECM) pathways underscores the significance of tumor–stroma interactions in facilitating invasion, angiogenesis, and metastasis. Future research utilising larger, multi-omics datasets combined with detailed clinicopathological and longitudinal outcome data will be vital for validating these candidate biomarkers and pathways. These strategies will be crucial for progressing precision oncology, allowing better risk stratification, early detection, and the development of targeted treatments for pancreatic cancer.

Supplementary materials

The Supplementary information includes figures:

• Supplementary Figure 1. Relative expression levels of the top four upregulated genes across different tumor stages compared to normal stages, based on data from the UALCAN database;

• Supplementary Figure 2. Relative expression levels of the top twelve downregulated genes across various tumor stages compared to normal stages, utilizing data sourced from the UALCAN database;

• Supplementary Figure 3. Depicts the (a) Biological Process, (b) Cellular Component, (c) Molecular Function, and (d) Pathway Analysis of upregulated genes. The X-axis shows ratios of differentially expressed genes to the total number of genes within each specific pathway. The color and size of the dots indicate the significance and number of genes enriched in each pathway, respectively;

• Supplementary Figure 4. Depicts the (a) Biological Process, (b) Cellular Component, (c) Molecular Function, and (d) Pathway Analysis of downregulated genes. The X-axis indicates ratios of differentially expressed genes to the total number of genes within each pathway. The color and size of the dots correspond to the significance and extent of gene enrichment in each pathway.

This supplemental material has been provided by the authors to give readers additional information about their work.

The file can be accessed using: https://www.editorialpark.com/download/article-supp/740/Supplementary-revised-copy.docx.

Author Contributions: Conceptualization, I.H.M. and K.A.; methodology, S.R. and I.H.M. ; validation, S.R., I.H.M., R.M. and K.A.; investigation, S.R. and C.A.S.; data curation, S.R. and C.A.S.; Software, S.R.; writing – original draft preparation, S.R.; writing – review and editing, I.H.M., R.M., M.E.S. and K.A.; visualization, S.R. and K.A.; supervision, K.A; funding acquisition, none. All authors have read and agreed to the published version of the manuscript.

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Identification of Early Genetic Markers in the Peripheral Blood of Newborns with Bronchopulmonary Dysplasia Using Bioinformatics Analysis and Machine Learning Algorithms

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ABSTRACT

Objective: To identify and characterize a unique set of biomarker genes in peripheral blood on day 5 of life in preterm infants with subsequent development of bronchopulmonary dysplasia.

Methods: The open dataset GSE32472 was used to analyze gene expression profiles. The sample included preterm infants with a gestational age of less than 32 weeks and a body weight of ≤1500g. Peripheral blood samples collected within a 5-day window were analyzed to identify early transcriptional changes. Differential gene expression analysis was performed using the limma package with thresholds of |log_2FC|>0.5 and p.adj<0.05. Coexpression network analysis and functional enrichment were used to narrow the pool of potential target genes. At the final stage, three independent machine learning algorithms, such as SVM-RFE, LASSO, and Random Forest, were used to select the most prognostically significant genes. The diagnostic model based on the selected genes was visualized as a nomogram, and its predictive ability was assessed using ROC analysis and decision curve analysis.

Results: Comparative analysis identified 451 differentially expressed genes in the BPD group on day 5 of life. WGCNA analysis identified the MEpink module as the one most significantly correlated with the BPD phenotype. The intersection of DEGs and genes within the MEpink module identified 106 common genes, functional analysis of which revealed significant enrichment for processes associated with T-cell activation and T-cell receptor signaling pathways. A combined machine learning approach reliably identified four key hub genes with high prognostic significance: BTN3A3, AK5, NOG, and GCSAM. A diagnostic model based on these four genes demonstrated high predictive ability (AUC = 0.888) and clinical utility. Regulatory analysis revealed that the AK5, BTN3A3, and GCSAM genes are central nodes in the miRNA–mRNA regulatory network.

Conclusion: The identified day 5 gene window reflects key pathophysiological processes: developmental impairment, cellular stress, and inflammatory immune dysregulation. These results shift the focus from BPD diagnosis to early risk prediction. Day 5 molecular signatures possess high prognostic value and can be used to develop targeted therapeutic interventions.

Keywords: bronchopulmonary dysplasia, bioinformatics analysis, machine learning, hub genes, preterm infants.

Introduction

Bronchopulmonary dysplasia (BPD) remains a leading pulmonary complication of prematurity, resulting in persistent respiratory symptoms and significant morbidity. The pathogenesis of "new BPD" is multifactorial, with key processes such as alveolar arrest and vascular dysgenesis beginning soon after birth [1-3]. Early identification of a high risk for developing BPD is critical for timely intervention before irreversible remodeling occurs.

Recent bioinformatic studies, such as the work of Luo et al. [4], have identified diagnostic biomarkers using the 28-day criterion. However, this criterion is based on the operational clinical definition of BPD [5-7] and reflects the chronic phase of the disease, characterized by remodeling.

Moreover, the pathogenesis of BPD is a dynamic process, beginning with acute injury to the immature lungs during the first week. Key drivers during this period are the acute inflammatory response, oxidative stress, and epithelial damage [2,8,9]. Therefore, it can be assumed that genes altered in the first week after birth should predominantly reflect molecular pathways associated with the acute immune response and mechanisms of cellular injury. These early genetic signatures will serve as true risk predictors, unlike markers at day 28, which are markers of already developed chronic pathology.

Objective of the study: To identify and characterize a unique set of biomarker genes in peripheral blood on day 5 of life in preterm infants with subsequent development of bronchopulmonary dysplasia.

Methods

2.1. Data sources

The open dataset GSE32472 from the Gene Expression Omnibus (GEO) database was used to analyze gene expression profiles. Primary clinical data for this dataset were collected at the Polish-American Children's Hospital from September 2008 to November 2010.

The study included preterm infants with a gestational age of less than 32 weeks and a birth weight of ≤1500 g who required respiratory support. Peripheral blood samples collected during the first 5 days of life (days 4 to 6) were chosen for analysis, allowing us to focus on early transcriptional changes that occur before the clinical signs of BPD.

The final dataset comprised data from 97 newborns, including 62 infants with BPD and 35 premature infants without signs of the disease. Pre-processing and normalization of the microarray data were carried out in R (version 4.3.2). The ggplot2 package was used to visualize the normalization results.

2.2. Identification of Differentially Expressed Genes (DEGs)

Differential gene expression was analyzed between the BPD and control groups using the limma package. Genes were considered differentially expressed if they had an absolute log_2-fold change $|log\_2FC| > 0.5$ and an adjusted p-value < 0.05. The results were then visualized with Volcano plots generated using the ggplot2 package.

2.3. Screening of target genes using WGCNA

Weighted Gene Co-expression Network Analysis (WGCNA) was performed using the WGCNA R package (version 1.72) to identify modules of co-expressed genes associated with bronchopulmonary dysplasia (BPD). After quality control to remove low-quality genes and samples, a signed co-expression network was constructed.

The soft-thresholding power was selected using the scale-free topology criterion, and a value of $\beta = 13$ was chosen as it achieved a signed scale-free topology fit index (R^2) greater than 0.8 while maintaining adequate mean connectivity. An adjacency matrix was calculated and transformed into a topological overlap matrix (TOM). Gene modules were identified using a dynamic tree-cutting algorithm implemented in the blockwiseModules function, with a merge cut height of 0.25 to merge similar modules.

Module eigengenes (MEs), defined as the first principal component of each module, were computed to represent the overall expression pattern of each module. Pearson correlation analysis was then performed between module eigengenes and clinical traits, including BPD status.

The MEpink module was prioritized for downstream analyses because it showed the strongest and most significant correlation with BPD. Genes within this module were intersected with differentially expressed genes to identify candidate genes potentially involved in BPD pathogenesis.

2.4. Functional enrichment analysis

To interpret the biological functions and pathways associated with overlapping genes—obtained at the intersection of DEGs and modules identified by the WGCNA method—functional enrichment analysis was performed. Enrichment by GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) categories was performed using the "clusterProfiler" package, with a statistical significance level of $Padj < 0.05$.

GO annotations were divided into three functional levels: biological processes (BP), cellular components (CC), and molecular functions (MF).

2.5. Construction of a protein-protein interaction network

To analyze the relationships between candidate genes, a protein–protein interaction (PPI) network was constructed using the STRING online platform (https://string-db.org/). The network was constructed using 106 genes identified at the intersection of DEGs and genes belonging to the most significant module, as determined by WGCNA analysis.

The confidence score was set at 0.4, which corresponds to a moderate degree of association reliability. Visualization of the resulting network and subsequent data processing were performed using Cytoscape (version 3.9.1).

2.6. Machine learning algorithms for biomarker screening in neonates with BPD

To select the most informative genes potentially associated with the development of BPD, three independent machine learning algorithms were used: SVM-RFE (Support Vector Machine – Recursive Feature Elimination), LASSO regression (Least Absolute Shrinkage and Selection Operator), and Random Forest (RF). To account for class imbalance within the cohort, model development and validation for SVM-RFE and LASSO were performed using stratified 10-fold cross-validation, ensuring that the original proportion of BPD cases and controls was maintained within each fold.

The SVM-RFE algorithm was used to identify the minimum subset of genes that provided the highest discriminative power. The optimal number of features was determined based on the maximum area under the ROC curve (AUC) across the stratified

folds. LASSO regression was implemented using the glmnet R package to perform regularization and feature selection. The optimal regularization parameter was selected based on the minimum value of the cross-validated error function, using AUC as the primary evaluation metric. Only genes with non-zero regression coefficients were retained for further analysis.

The third model, Random Forest, was trained on the full dataset to estimate the contribution of each gene to the phenotype classification. Feature importance was derived using the Mean Decrease Gini (impurity measure), which provides a robust estimation of variable significance in the presence of moderate class imbalance. The intersection of the gene sets identified by all three algorithms was considered the final set of reliable candidate biomarkers. This overlapping subset was then used for the construction of the final prognostic model, with the results visualized using a Venn diagram.

2.7. Construction of a nomogram model

To visualize the predictive model, a nomogram was constructed using logistic regression, incorporating the selected genes as predictors (rms package). The model was calibrated using bootstrap resampling (B = 1000), with a calibration curve plotted demonstrating the agreement between the predicted and observed probabilities. The diagnostic performance of the model was assessed using receiver operating characteristic (ROC) analysis, where the area under the curve (AUC), reflecting the classification accuracy between groups, was calculated. Additionally, Decision Curve Analysis (DCA) was performed to evaluate the clinical utility of the model and the range of threshold probabilities at which the use of the predictive model provides the greatest net benefit compared to the "treat all" and "treat no one" strategies.

2.8. Potential mechanisms of biomarker regulation

To investigate post-transcriptional regulatory mechanisms associated with bronchopulmonary dysplasia, miRNA–mRNA interactions were identified using the multiMiR R package. Candidate genes obtained from integrative differential expression, WGCNA, and machine-learning analyses were queried against multiMiR. Only validated interactions were retained by restricting the query to the validated multiMiR table, which integrates curated evidence from miRTarBase, TarBase, and miRecords. Computationally predicted interactions were excluded from the final network to ensure high-confidence regulatory relationships. All interactions were filtered to include only Homo sapiens targets and miRNAs with standardized mature miRNA annotations. The resulting miRNA–mRNA interactions were visualized as a regulatory network, and a complete list of validated interactions, including the supporting database source, is provided in Supplementary Table 1.

Results

3.1. Characteristics of the study population

In the present study, data from 97 patients were analyzed. Bronchopulmonary dysplasia was diagnosed in 62 (64%) participants. The cohort's demographic characteristics, stratified by the presence or absence of BPD in the 5-day window (days 4–6), are presented in Table 1. A statistically significant difference was observed between the groups. Patients with BPD had a lower mean gestational age at birth (26.1 weeks vs. 30 weeks; $P < 0.0001$) and a lower mean birth weight (802 g vs. 1280 g; $P < 0.0001$). Of the 62 patients with BPD, 15 had a severe form. The mean birth weight and gestational age in this subgroup were 690 g and 25 weeks, respectively.

3.2 Detection of DEG in patients with bronchopulmonary dysplasia

After standardization and normalization of peripheral serum samples, comparative gene expression analysis between the BPD group and the control group resulted in the identification of 451 differentially expressed genes. Volcano plot analysis revealed that of the total number of DEGs, 296 were upregulated and 155 were downregulated in BPD patients on day 5 of life (Figure 1).

3.3 Overlapping genes between genes of BPD-associated modules with DEGs

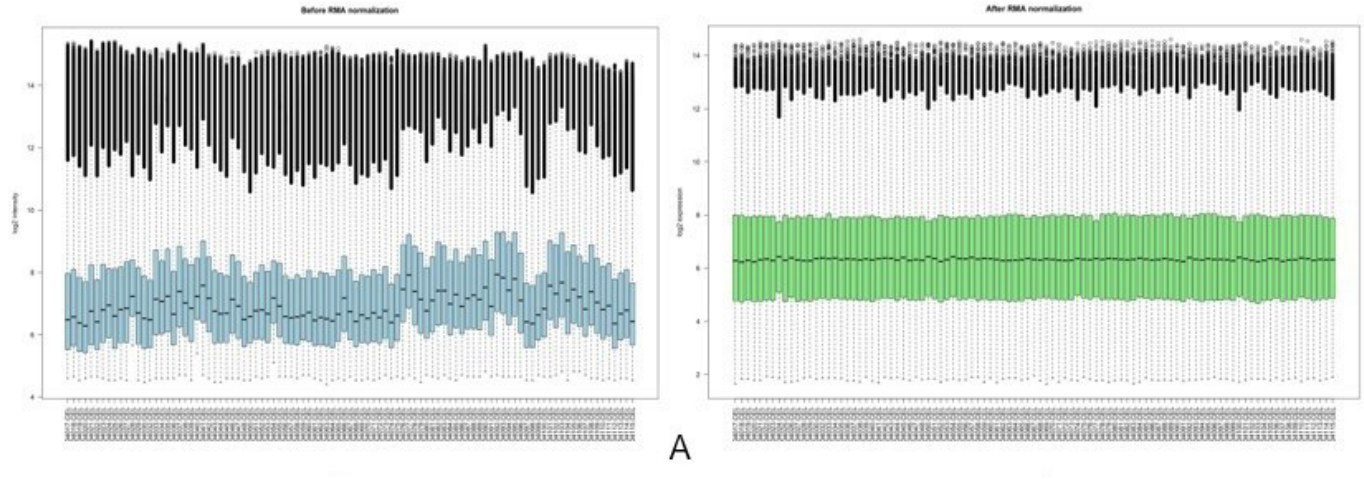
Using weighted gene co-expression network analysis (WGCNA), several gene modules were identified that showed statistically significant correlation with the BPD phenotype. The strongest correlations with BPD were found for the modules MEpink=-0.54, MEgreenyellow=0.45, MEblack=0.43, and MEgrey60=-0.4 (Figure 2A). To narrow the pool of genes with the greatest pathogenetic significance, an overlap analysis was performed between the identified DEGs and the genes included in the module with the most significant correlation (MEpink). This analysis resulted in the identification of 106 common genes, which accounted for 15.4% of the total number of genes included in the analysis (Figure 2B). These genes were then used to construct a protein-protein interaction (PPI) network using the STRING database. The resulting PPI network was characterized by 76 nodes and 621 edges, indicating a high degree of interaction and functional connectivity between proteins (Figure 2C).

3.4. Functional enrichment analysis

Functional analysis performed on the overlapping genes revealed significant enrichment for immune-related processes (Figure 3). The dominant biological process categories were T-cell activation, T-cell receptor signaling pathways, lymphocyte differentiation, and various immune response-regulating pathways. Among the cellular component categories, enrichment was found for T-cell receptor complexes, the outer plasma membrane, and the immunological synapse. Molecular function categories included T-cell receptor binding, signaling adaptor activity, and purinergic receptor activity.

Table 1 Characteristics of the study group

Variables	Total (n=97)	Control (n=35)	Mild (n=34)	Moderate (n=13)	Severe (n=15)	P value
Gestational age	28(4)	30(6)	26,5(9)	27(8)	25(9)	<0,0001
Birth weight	1000(508)	1280(750)	915(790)	800(920)	690(480)	<0,0001
ROP	47(48,5%)	2(5,7%)	21(61,7%)	9(69,2%)	15(100%)	<0,0001
PVL	14(14,4%)	1(2,8%)	7(20,5%)	2(15,3%)	4(2,7%)	0,0833
Female	45(46,4%)	21(60%)	13(38,2%)	5(38,4%)	6(40%)	0,2518



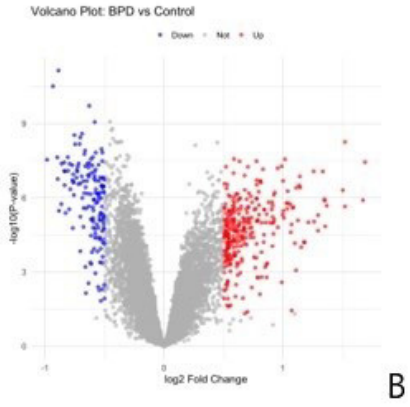
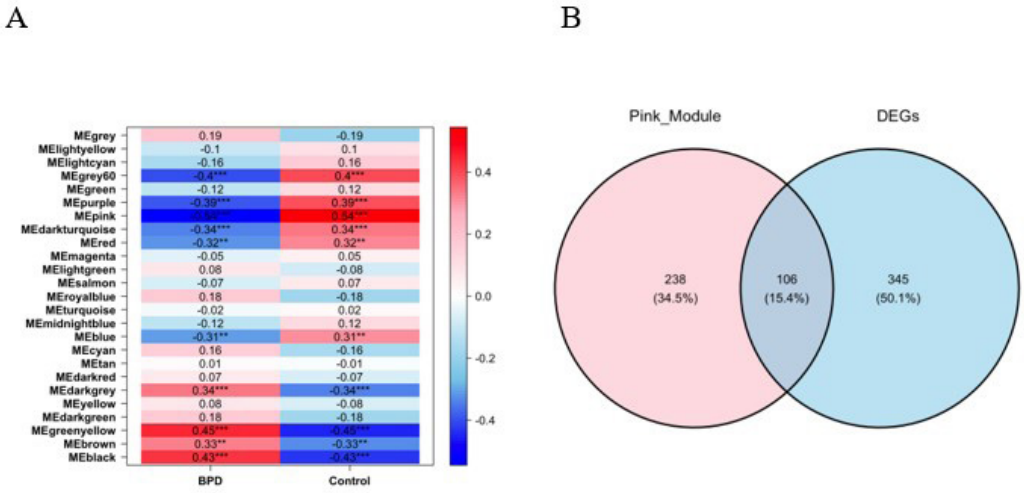


Figure 1 – Identification of differentially expressed genes in bronchopulmonary dysplasia. (A) Normalization of expression data across samples was applied. (B) Volcano plot displays DEG expression patterns: upregulated genes are highlighted in red, and downregulated genes are highlighted in blue.



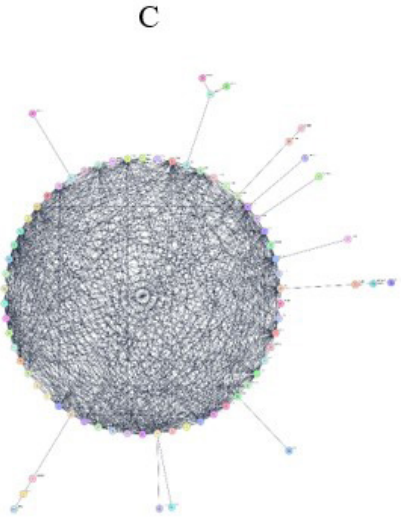


Figure 2 – Identifying the most important modules using WGCNA. A - Correlations between modules and traits, B - Venn diagram showing overlapping genes, C - PPI network of overlapping genes.

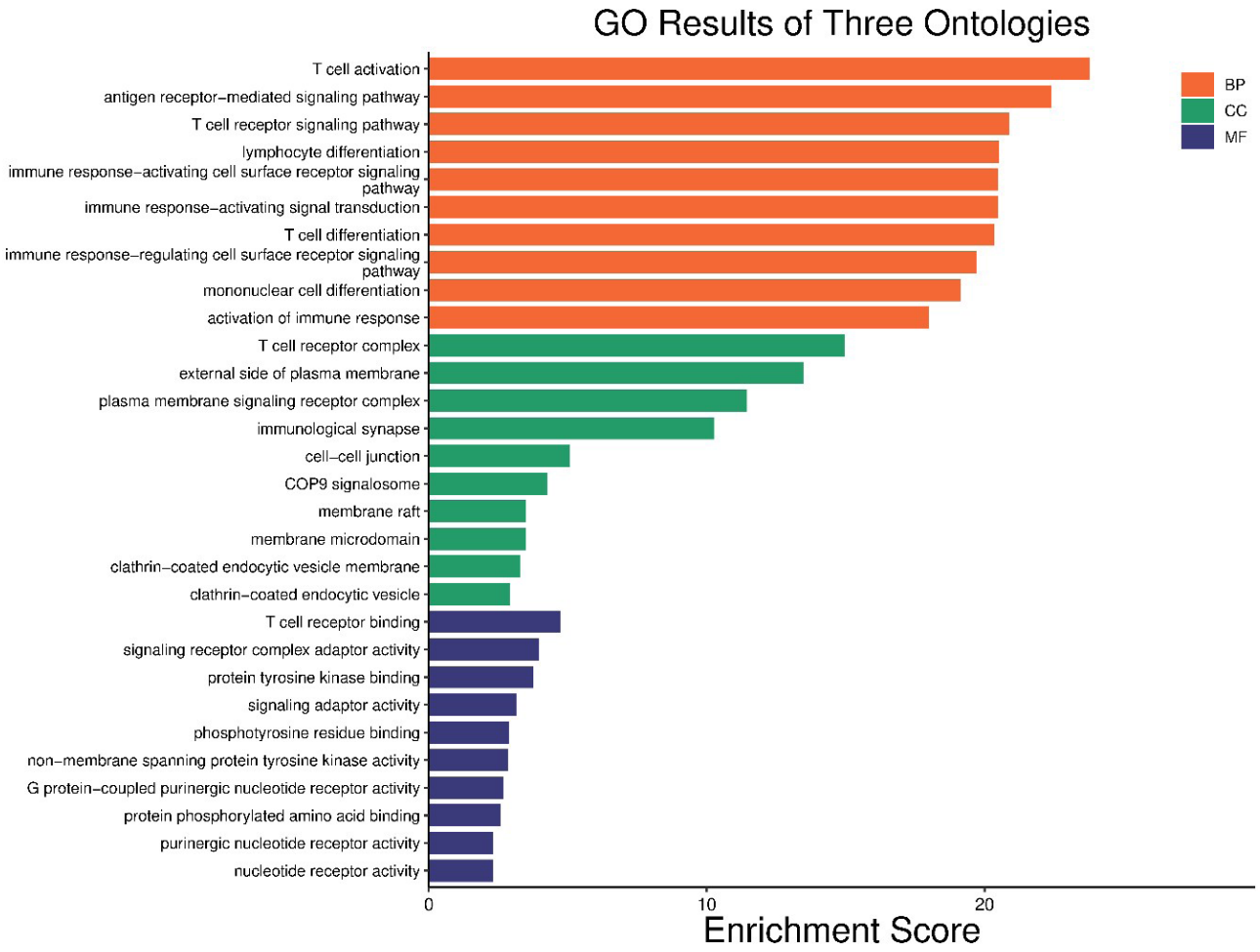


Figure 3 – Functional enrichment of overlapping genes by GO

KEGG pathway analysis confirmed the involvement of genes in immune regulation, particularly T cell receptor signaling pathways, Th1 and Th2 cell differentiation, as well as Th17 cells and cell adhesion (Figure 4).

3.5. Identification of the hub gene

To identify gene signatures with the highest prognostic value, a combined approach using three machine learning algorithms was used: SVM-RFE, LASSO, and Random Forest. The SVM-RFE model demonstrated the highest accuracy of 0.82 using the minimum feature set. This method identified six gene signatures (Figure 5A). LASSO regression analysis identified 11 gene signatures (Figures 5B and C), while the random forest method identified 20 genes with importance scores (Figures 5D and E). Joint analysis of the results of the three algorithms allowed us to confidently identify four common hub genes, which were obtained by overlapping genes from the three methods. These hub genes are BTN3A3, AK5, NOG, and GCSAM (Figure 5F).

3.6. Construction of a diagnostic model

Based on these four hub genes, a diagnostic model for predicting the risk of BPD was developed. ROC analysis demonstrated high predictive ability with an AUC of 0.888. Model reliability was confirmed by a calibration curve, which demonstrated good agreement between the predicted and observed outcomes. Integration of BTN3A3, AK5, NOG, and GCSAM gene expression levels was implemented using a nomogram for individual quantitative assessment of BPD risk. The clinical utility of the model was confirmed by DCA, which showed a consistent increase in net benefit over a wide range of threshold probabilities (Figure 6).

3.7. Mechanisms of potential biomarkers regulation

To further explore potential post-transcriptional regulatory mechanisms influencing the expression of the identified hub genes with high prognostic value, a bioinformatics analysis was conducted to construct a miRNA-mRNA interaction network. The resulting network exhibits a complex hierarchical architecture and is characterized by a high degree of connectivity, indicating multiple regulatory loops. The biomarker genes AK5, BTN3A3, and GCSAM act as central hubs, with the AK5 gene demonstrating the most extensive regulatory loop and being targeted by the largest number of miRNAs, emphasizing its critical role in cellular response. However, the NOG gene was not included in this subnetwork, indicating the possible prevalence of other regulatory mechanisms. Visually, the network confirms the presence of cross-regulation, as each of the three hub genes is regulated by a significant number of unique and partially shared miRNAs (Figure 7).

Discussion

Bronchopulmonary dysplasia (BPD) remains a critical pulmonary complication of prematurity. The modern concept of "new" BPD has shifted from the classical barotrauma model to a dynamic disease characterized by arrested alveologenesis and pulmonary vascular dysgenesis against a background of lung immaturity [5]. The pathogenesis is biphasic and cascading,

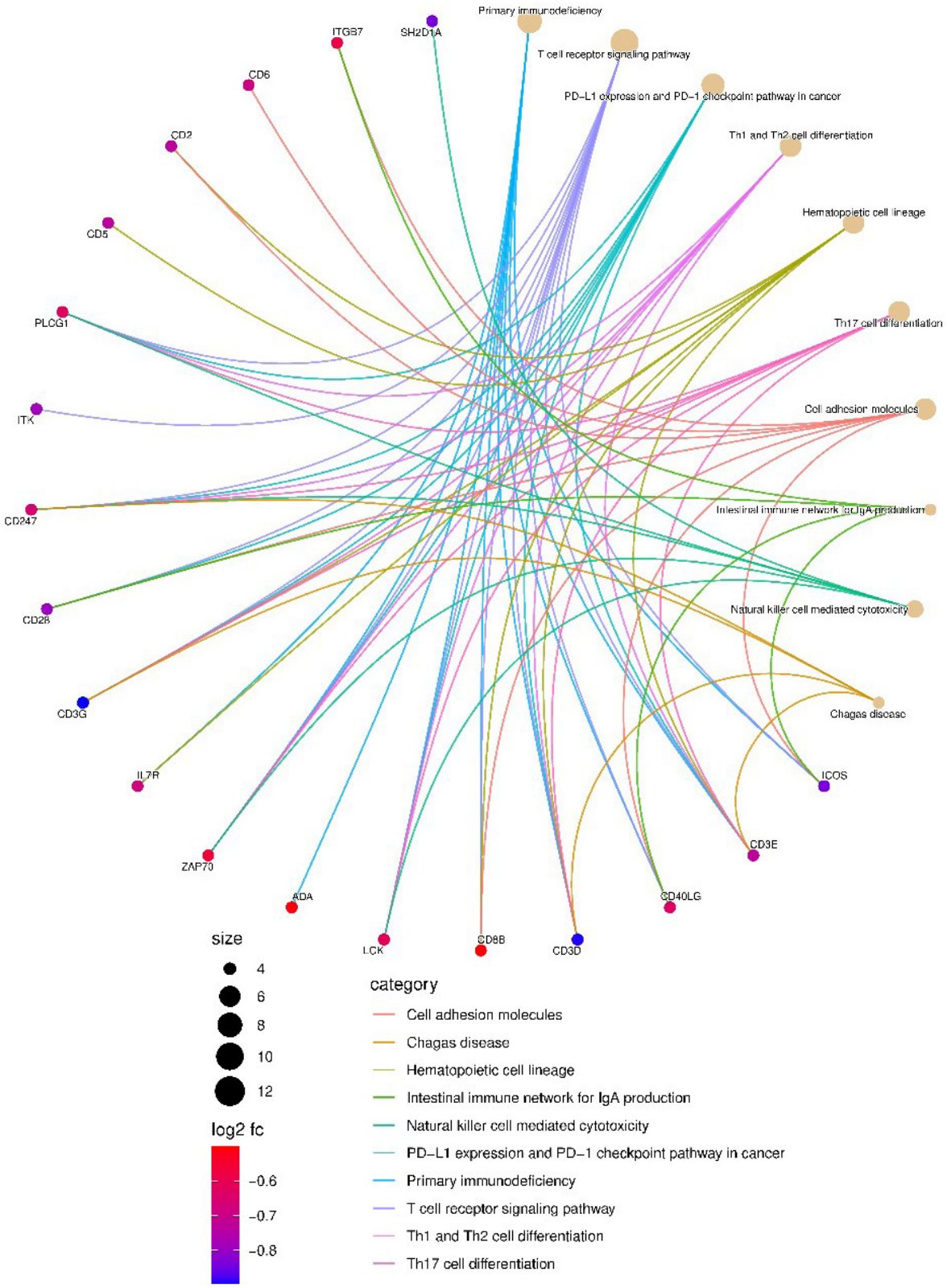


Figure 4 – Functional enrichment of overlapping genes by KEGG

with injury and inflammation dominating the acute phase and impaired regeneration and remodeling in the chronic phase [6]. This underscores the urgent need to develop early prognostic biomarkers in the first days of life.

Our findings, identifying the AK5, NOG, GCSAM, and BTN3A3 genes as potential biomarkers of BPD at day 5 after birth, support the hypothesis of a dynamic nature of early pathogenesis. For consistency with previous studies, particularly the work of Luo et al. [4], we used the same highly informative GSE32472 dataset. However, unlike these authors, who focused on day 28, we applied differential expression analysis, WGCNA, and machine learning algorithms to a sample limited to a 5-day time window. This critical time shift allowed us to identify new hub genes with high prognostic value in the early, acute phase of the disease.

Identification of hub genes in peripheral blood at such an early stage is crucial because BPD is not only a local lung disease but also a systemic inflammatory condition [10,11].

Accordingly, genetic markers in the blood reflect the body's global immune and metabolic response to acute lung injury

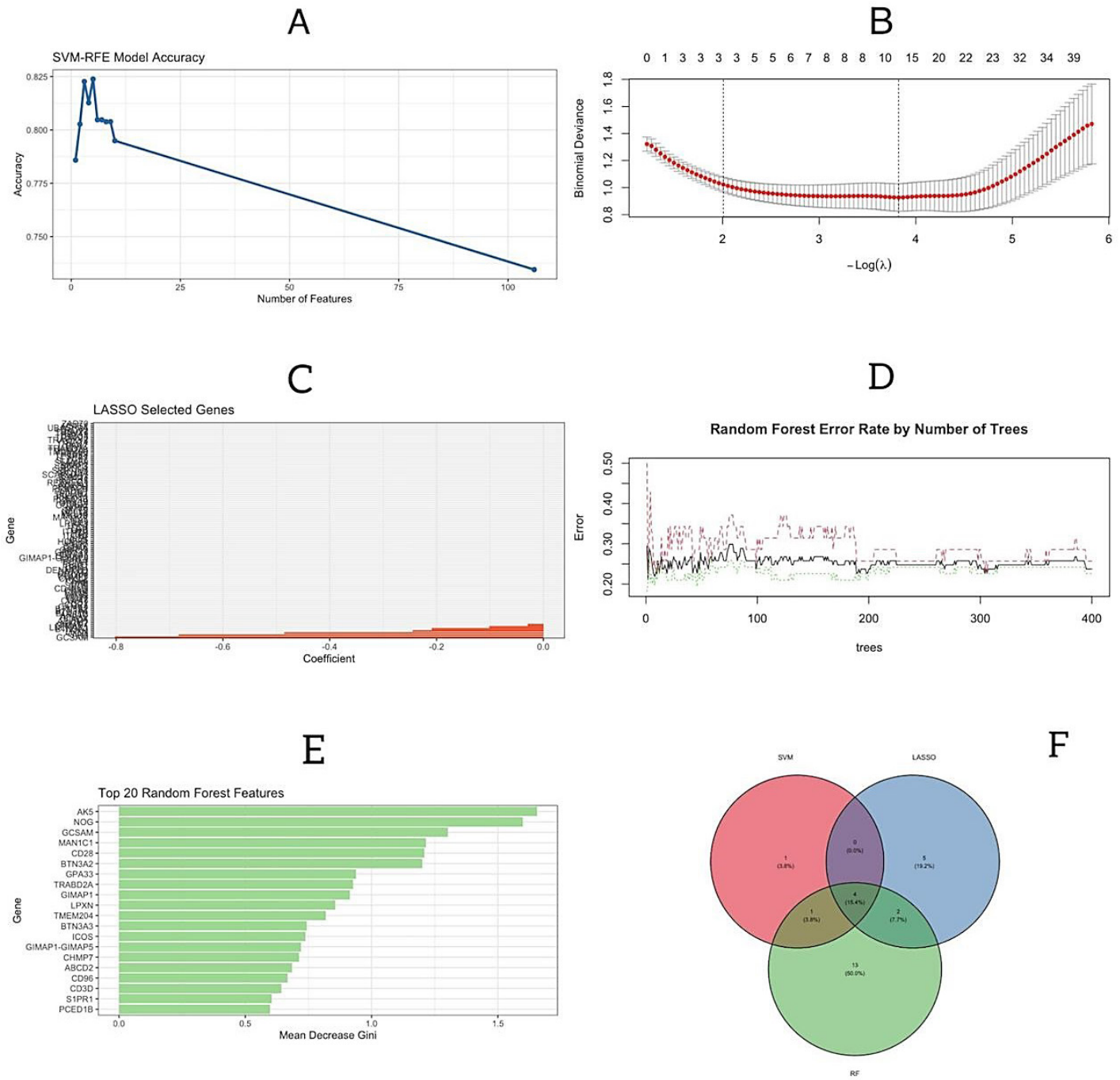


Figure 5 – Screening of potential biomarkers using a machine learning approach. A - SVM-RFE analysis, B - candidate gene coefficient profiles obtained from LASSO regression analysis, C - important features selected by LASSO, D - RF model prediction accuracy, E - RF model gene importance score, F - Venn diagram showing the 4 hub genes common to the 3 machine learning algorithms

and immaturity. These molecular signatures serve as a readily available surrogate for early pathophysiological processes, such as celluar stress and inflammatory cell recruitment, which precede irreversible remodeling.

Unlike diagnostic markers of the chronic phase (day 28), our predictive markers of day 5 reflect key pathways associated with acute lung injury and early disruption of the developmental program. Identifying these hub genes during the critical window of the acute phase has crucial prognostic and therapeutic implications.

A key finding is the involvement of the NOG (Noggin) gene, a high-affinity antagonist of the bone morphogenetic protein (BMP) signaling pathway, a central regulator of lung morphogenesis [12–14]. Dysregulation of the BMP/Noggin pathway is associated with impaired alveologenesis and vascular dysgenesis, which is the morphological basis of "new" BPD [15]. Changes in NOG expression as early as day 5 may indicate that a critical disruption of the developmental program is triggered immediately after acute injury, acting as an early molecular predictor of alveolar arrest.

The BTN3A3 (Butyrophilin Subfamily 3 Member A3) gene has been identified as a critical component linking the genetic signature to inflammatory pathogenesis, as evidenced by the association of its polymorphisms with an increased risk of BPD. As a member of the butyrophilin family, BTN3A3 is functionally involved in the regulation of T-cell responses, primarily through modulation of γδ cells [16–18]. Detection of BTN3A3 in circulating blood on day 5 highlights that the acute inflammatory cascade and immune activation rapidly translate into pathological processes, making BTN3A3 a marker of the intensity of the acute immune response in BPD.

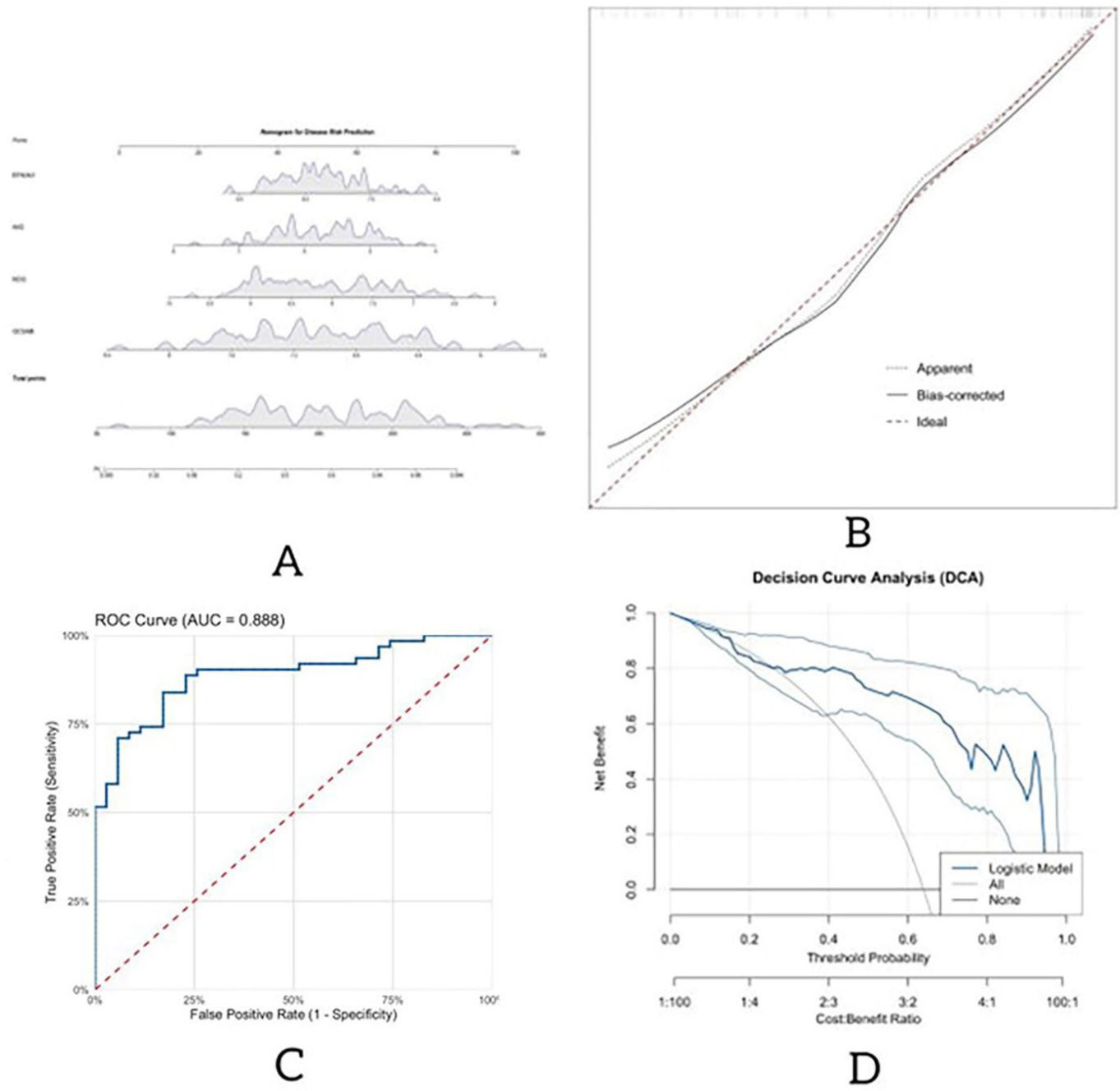


Figure 6 – Nomogram model for diagnosing BPD. (A) Nomogram for predicting the risk of BPD. (B) Diagnostic performance of the nomogram model was assessed using calibration curves. (C) Area under the curve of the nomogram model. (D) Practical performance of the nomogram using DCA curves

The AK5 (Adenylate Kinase 5) gene, a key enzyme in energy homeostasis via adenosine phosphate transphosphorylation, functionally reflects critical cellular stress and injury. The acute phase of BPD, characterized by hyperoxia, ischemia-reperfusion, and inflammation, initiates mitochondrial dysfunction and energy deficit. Failure of AK5-related energy metabolism control systems is an integral part of the injury cascade, as evidenced by the critical role of AMP-α in endothelial protection [19–21]. Alterations in AK5 expression on day 5 may reflect molecular evidence of systemic impairment of cellular viability and a metabolic shift.

GCSAM (Germinal Center Associated Signaling and Motility), an adapter protein, regulates the B-cell receptor (BCR) signaling pathway and lymphocyte motility, particularly through RhoA activation [22]. Elevated GCSAM levels in peripheral blood may reflect early activation and mobilization of immune cells in response to lung injury. Since its expression is induced by interleukin-4 (IL-4) [23], GCSAM may serve as an early signaling beacon of IL-4-dependent immune dysregulation and potentially reflects the systemic regulation of cellular infiltration in the acute phase of BPD.

The conducted analysis of the miRNA–mRNA network is a critical complement to the differential expression data, as it reveals post-transcriptional regulatory mechanisms underlying the acute phase of BPD. The hierarchical architecture of the network, with the AK5, BTN3A3, and GCSAM genes acting as central hubs, confirms their key role in early pathogenesis. The fact that their expression is actively targeted by multiple miRNAs indicates that they are tightly regulated nodes highly sensitive to systemic inflammatory signals.

The exclusion of the NOG gene from this miRNA-mRNA subnetwork is logically consistent with its primary role in morphogenesis and development [24], where its expression is under the predominant control of epigenetic mechanisms

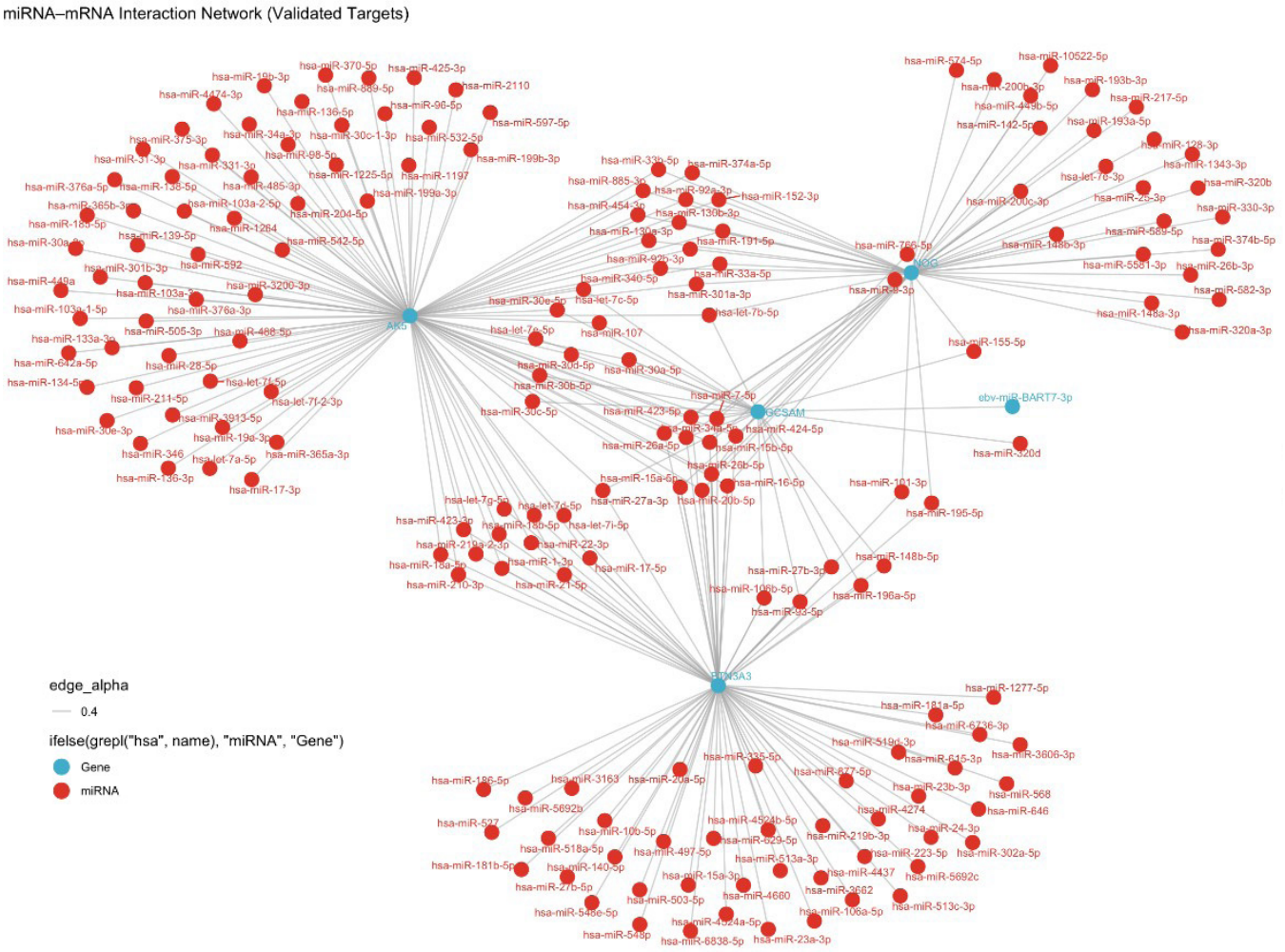


Figure 7 – Mechanisms of potential biomarkers regulation

and transcription factors rather than rapid post-transcriptional modulation. Thus, NOG reflects a disruption of the developmental program, while AK5, BTN3A3, and GCSAM reflect a dynamic cellular response to systemic stress controlled by miRNAs.

A key limitation of the study is its retrospective nature, based on a secondary analysis of the publicly available GSE32472 dataset. Detection of differential gene expression in peripheral blood serves as a surrogate marker of systemic response, which does not allow for direct assessment of pathophysiological processes in situ within the lung parenchyma. The lack of experimental validation precludes establishing a causal relationship between altered gene expression and the development of BPD. External testing in independent prospective cohorts of preterm infants is required to confirm the high prognostic value and clinical applicability of the identified marker set.

Furthermore, the uneven group sizes remain a significant limitation. Although we used stratified cross-validation for SVM-RFE and LASSO models to maintain class proportions and prioritized the area under the ROC curve, the absence of explicit resampling techniques, such as oversampling or synthetic data generation, means our results should be interpreted with caution. The study is also constrained by its reliance on a single-center cohort.

The analysis is further restricted by the absence of granular clinical covariates within the dataset, such as specific ventilation parameters, cumulative oxygen exposure, and the presence of neonatal infections, which are known to influence both gene expression and BPD progression. Additionally, although samples were restricted to a five-day window (days 4 to 6), the inherent temporal heterogeneity in sample collection during this acute phase may introduce variability in transcriptional signatures. Finally, while we identified four hub genes with high predictive potential, the lack of prospective validation means these findings serve as a foundation for future research. Future studies using larger, more balanced prospective cohorts and implementing class-weighted learning strategies will be essential to validate these markers and confirm their clinical utility in diverse neonatal populations.

Conclusion

Despite these limitations, the identified day 5 gene window reflects key pathophysiological processes: developmental impairment, cellular stress, and inflammatory immune dysregulation. These results shift the focus from BPD diagnosis to early risk prediction. Day 5 molecular signatures possess high prognostic value and can be used to develop targeted therapeutic interventions. Further functional studies of these genes in BPD models are necessary to validate their role as therapeutic targets.

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Data availability statement: The corresponding author can provide the data supporting the study's conclusions upon request. Due to ethical and privacy constraints, the data are not publicly accessible.

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Depression Prevalence in Urban Indonesia: a Preliminary Study of the Makassar General Population

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ABSTRACT

Introduction: The prevalence of depression in South Sulawesi has been reported to exceed the national average. This preliminary study investigated the prevalence of depression in Makassar, the capital city of South Sulawesi.

Methods: A total of 64 volunteers from the general population were enrolled for cross-sectional observational study using convenience sampling. The CES-D questionnaire was used to assess depressive symptoms, and sociodemographic data were also collected. The main analysis was conducted using a generalized linear model (GLM).

Results: The results showed that the mean CES-D score was 16.34 ± 10.30, and the prevalence of screen-positive mild-to-severe depressive symptoms was 65.6%. CES-D scores varied significantly by age (p = 0.026), ethnicity (p = 0.004), and education (p = 0.041).

Discussion: However, in the multivariate analysis, ethnicity remained statistically associated with depressive symptoms, with participants of Makassar ethnicity observed to report lower levels of depressive symptoms compared with other ethnic groups. This preliminary finding may suggest a context-specific association between cultural identity and lower depression scores.

Conclusion: These findings provide preliminary information that requires further investigation through large-scale observational studies to examine the association between ethnicity and depression, particularly in urban settings characterized by emerging multiethnic identities.

Keywords: Depression, Ethnicity, Makassar, Indonesia, Urban mental health

Introduction

Indonesia has been widely reported to have a substantial prevalence of depression, as evidenced by numerous observational studies conducted across various populations and regions of the country [1–10]. The prevalence reported is relatively high and, in some cases, comparable to or even exceeding estimates from other Southeast Asian countries [11,12].

The prevalence of depression in Indonesia is generally reported at the provincial level [13,14], with limited data available at the city level, particularly in urban settings. Makassar, as one of the rapidly growing metropolitan cities in South Sulawesi, represents an important context for examining depression prevalence. Despite this, research on depression in South Sulawesi—particularly in Makassar—remains limited. The rapid acceleration of urbanization, shifts in the sociocultural landscape, and increasing demographic diversity accompanying the city's development may offer valuable perspectives for understanding the emerging patterns of depressive symptoms within this urban population [2,13,15,16].

Depression has been widely investigated in relation to sociodemographic (SD) characteristics [17,18], including within culturally and demographically diverse populations in Indonesia [1–3,7–9,11]. For instance, findings from studies conducted in Makassar indicate that income is significantly associated with

depressive symptoms [19], whereas no meaningful gender differences have been identified [20]. The authors of these studies suggest that such associations may be shaped by broader sociocultural dynamics inherent to urban environments, including rapid urbanization, evolving cultural norms, and growing demographic heterogeneity. This perspective is further supported by research examining self-construal and depressive symptoms among residents of Makassar [21], which indicates that cultural orientations may influence how depressive symptoms are experienced, interpreted, and expressed within this urban population.

Therefore, this study was designed as a preliminary investigation to examine the prevalence of depressive symptoms within an urban context, using Makassar City as a case example. In addition, this study aimed to explore the association between sociodemographic factors and depression scores among urban residents. Based on the evidence from previous studies, it is assumed that the manifestation of depressive symptoms in this population may be context-dependent, shaped by the unique sociocultural dynamics of urban living.

Methods

Study design and Setting

This was a preliminary observational study using a cross-sectional design conducted in Makassar City, South Sulawesi, Indonesia. The reporting of this study adhered to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (https://www.strobe-statement.org/). South Sulawesi is a province that has reported a comparatively high prevalence of depression [13,14], and as its capital, Makassar may contribute substantially to this estimate.

Participants

A total of 64 residents of Makassar voluntarily participated in this study. The sample was drawn from the general population using a convenience sampling method. The inclusion criteria required that participants had been residents of Makassar for at least 10 years and were a minimum of 15 years of age at the time of data collection. Eligible individuals were then invited to participate after receiving an explanation of the research objectives and procedures, and those who agreed provided written informed consent.

CES-D

Depressive symptoms were assessed using the Center for Epidemiologic Studies Depression Scale (CES-D) [22]. This instrument is valid and widely used for measuring depressive symptomatology in the general population [4,22–24]. The CES-D consists of 20 items assessing how frequently individuals experienced specific symptoms (e.g., "I was bothered by things that usually don't bother me") during the past week. Each item is rated on a 4-point Likert scale ranging from 0 (rarely or none of the time) to 3 (most or all of the time). The internal consistency reliability of the CES-D in this study was 0.80. Following the established scoring criteria, CES-D total scores were categorized into: no depression (0–9), mild depression (10–15), moderate depression (16–24), and major/severe depression (≥25) [24]. These cut-offs were selected to maintain comparability with prior Indonesian population studies using the CES-D [1,3,5].

Sociodemographic (SD)

Sociodemographic data included basic respondent information such as age, sex, ethnicity, educational attainment, occupation, and monthly household income. Age was categorized into four groups: 15–30, 31–45, 46–60, and 61–75 years. Educational attainment was classified into four levels: elementary school or below, secondary school, diploma, and bachelor's degree or higher. Occupational status included housewife, private-sector worker, and student; categories with low frequencies were combined and classified as "others." Monthly household income was categorized as <IDR 1,000,000; IDR 1,000,000–2,000,000; IDR 2,000,000–3,000,000; IDR 3,000,000–5,000,000; and IDR 5,000,000–7,500,000. Ethnicity was classified into major ethnic groups (i.e., Makassar, Bugis, and Toraja), mixed Bugis–Makassar ethnicity, and a minority category labeled "others." Any missing or unreported information was categorized as "unknown."

Procedures

Participants who provided consent then underwent a face-to-face interview conducted by a trained field enumerator, which lasted approximately 10–15 minutes. Prior to data collection, all field enumerators completed a standardized trainer-of-trainers (ToT) training to ensure they fully understood and properly executed study procedures, interviewing techniques, and data-collection protocols.

Data analysis

The reliability of the CES-D scale was assessed using Cronbach's alpha (α). Data were analyzed descriptively using proportions (n and %) and measures of central tendency (mean±standard deviation). The Mann–Whitney U test and Kruskal–Wallis test were used to examine differences in CES-D scores across sociodemographic groups. To evaluate the associations between sociodemographic variables and depressive symptom scores at the multivariate level, a generalized linear model (GLM) was applied. All statistical analyses were conducted using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). A 95% confidence interval (CI) and a significance level of $\alpha = 0.05$ were used to determine statistical significance.

Ethical approval

This study received ethical approval from the Health Research Ethics Commission of the Faculty of Public Health, Hasanuddin University (Protocol No. 31025105045).

Results

Table 1 (see the next page) shows that the mean CES-D score among participants was 16.34 ± 10.30. Based on established cut-off values, 34.4% of respondents were classified as having no depressive symptoms, while 23.4%, 18.8%, and 23.4% were classified as having mild, moderate, and major depressive symptoms, respectively. A smaller proportion of participants also demonstrated very high depressive symptom scores (CES-D >30; Figure 1). The mean age of participants was 30.73 ± 14.20 years, with individuals aged 15–30 years constituting the largest proportion of the sample (65.6%). Male and female participants were equally represented. Most participants identified as Makassar ethnicity (45.3%). The majority had completed secondary education (62.5%), were students or reported other occupations (34.4%), and reported a monthly income ranging from IDR 2,000,000 to 3,000,000 (21.9%).

Mean CES-D scores varied across sociodemographic characteristics (Table 2; Figure 2). Higher mean CES-D scores were observed among participants aged 15–30 years (18.33 ±

Table 1 Respondent characteristics

Parameter	Frequency (N=64)
CES-D score	16.34±10.30
CES-D category	
No depression (0-9)	22 (34.4)
Mild depression (10-15)	15 (23.4)
Moderate depression (16-24)	12 (18.8)
Major depression (≥25)	15 (23.4)
Age (years)	30.73±14.20
Age (category, years)	
15–30	42 (65.6)
31–45	12 (18.8)
46–60	6 (9.4)
61–75	4 (6.3)
Sex	
Male	32 (50.0)
Female	32 (50.0)
Ethnicity	
Bugis	13 (20.3)
Makassar	29 (45.3)
Bugis-Makassar	13 (20.3)
Toraja	1 (1.6)
Others	8 (12.5)
Education	
Elementary school or less	11 (17.2)
Secondary school	40 (62.5)
Diploma	1 (1.60)
Bachelor or higher	12 (18.8)
Occupation	
Housewife	16 (25.0)
Private	2 (3.1)
Students	22 (34.4)
Others	22 (34.4)
Unknown	2 (3.1)
Household income (IDR; Monthly)	
<1,000,000	11 (17.2)
1,000,000 – 2,000,000	10 (15.6)
2,000,000 – 3,000,000	14 (21.9)
3,000,000 – 5,000,000	8 (12.5)
5,000,000 – 7,500,000	2 (3.1)
Unknown	19 (29.7)

Data were presented as mean ± standard deviation and n (%). CES-D = Center for Epidemiologic Studies Depression Scale. Missing or unreported responses were recorded as "unknown."

10.82), males (17.43 ± 11.13), and those with secondary school education (17.52 ± 10.62). Participants identifying as Makassar ethnicity had the lowest mean CES-D score (12.72 ± 9.23) compared with other ethnic groups. Differences in CES-D scores across age categories, ethnicity, and educational attainment were statistically significant, whereas differences by sex, occupation, and household income were not statistically significant (Table 2 and Figure 2).

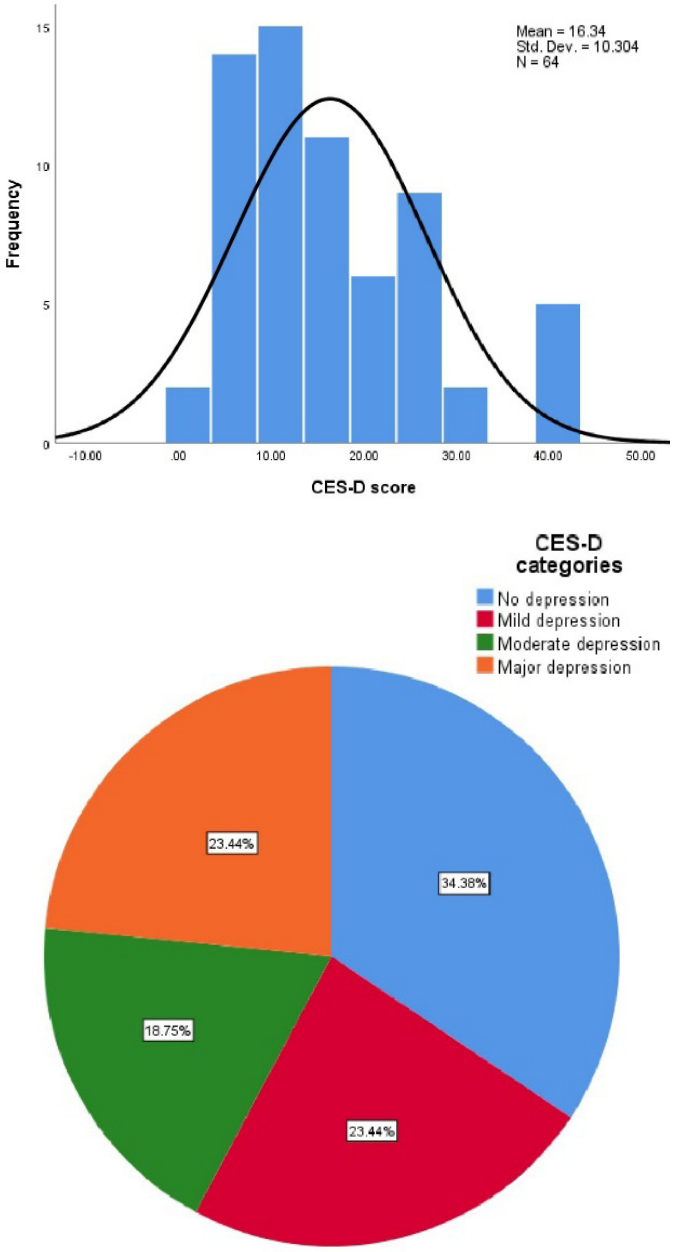

Figure 1 – CES-D mean score and categories

Table 3 presents the generalized linear model analysis examining the association between sociodemographic variables and CES-D scores. Ethnicity remained significantly associated with CES-D scores (p=0.014), whereas age, sex, educational level, occupational status, and income were not statistically associated with depressive symptom scores.

Discussion

This study represents a preliminary investigation of depression prevalence among residents of Makassar, using a small, exploratory sample of 64 participants to obtain an initial overview of depressive symptomatology within the population. The findings indicated a relatively elevated mean CES-D score (16.34±10.30), suggesting a substantial proportion of participants screened positive for depressive symptoms. Surprisingly, 65.6% of the sample fell within the mild-to-severe depression categories. These findings are comparable to those reported in previous studies with larger sample sizes that employed the same CES-D instrument in general population settings [7,20]. As such, the observed estimates may provide a rough approximation

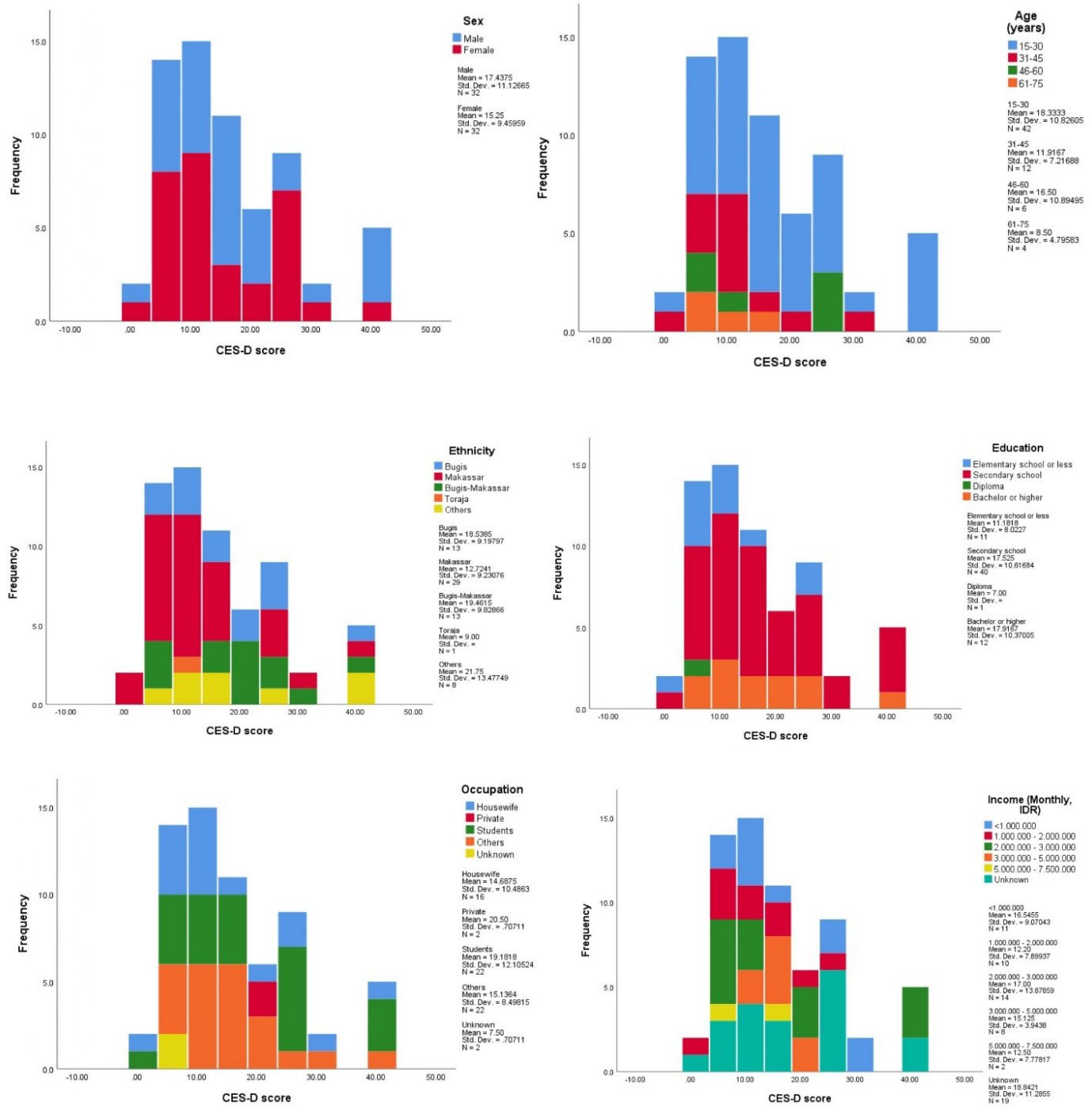

Figure 2 – Mean CES-D scores across respondent characteristics

of depressive symptom prevalence, despite being derived from a limited sample. However, these prevalence estimates should still be interpreted with caution. The use of convenience sampling may have influenced the demographic composition of the sample, particularly with respect to age and educational background, which may have resulted in overrepresentation within the estimates and limited the generalizability of the findings to the broader population. Moreover, the small sample size constitutes an important methodological limitation and should be carefully considered when interpreting the results. In addition, variability in CES-D cut-off scores across studies may contribute to differences in the classification and reported prevalence of depressive symptoms, further complicating direct comparisons with the existing literature [22–24]. For instance, when threshold ≥16 was applied [4], the prevalence decreased to 42.2%. Nevertheless, the presence of a substantial proportion of participants with mild depressive symptoms, based on the established cut-off points used in the current study, may provide a valuable signal of early vulnerability to depressive symptomatology.

The high prevalence of depressive symptoms (i.e., CES-D scores) observed in this study appears to be partly attributable to differences in age, ethnicity, and educational attainment (see Table 2 and Figure 2). These sociodemographic (SD) factors have been well documented in previous research as sources of variation in depression prevalence [1–3, 7–9, 11]. Variation in CES-D scores across age groups may reflect the sensitivity of the instrument in general population samples, particularly

Table 2 Mean comparation of CES-D based on sociodemographic

Parameter	CES-Da	p	CES-Db	p
Age (category, years)				
15–30	18.33±10.82	NS	18.33±10.82	0.026
31–45	11.91±7.21		12.54±8.16c	
46–60	16.50±10.89			
61–75	8.50±4.79			
Sex				
Male	17.43±11.13	NS		
Female	15.25±9.46			
Ethnicity				
Makassar	12.72±9.23	0.043	12.72±9.23	0.004
Bugis	18.53±9.20		19.34±10.30	
Bugis-Makassar	19.50±9.83			
Others	20.33±13.30			
Education				
Elementary school or less	11.18±8.02	NS	11.18±8.02	0.041
Secondary school	17.52±10.62		17.41±10.46	
Diploma or higher	17.07±10.37			
Occupation				
Students	19.18±12.10	NS	19.18±12.10	NS
Private	20.50±0.71		14.85±9.03	
Housewife	14.68±10.48			
Others	15.13±8.49			
Unknown	7.50±0.71			
Income (IDR; Monthly)				
<1,000,000	16.54±9.07	NS	16.54±9.07	NS
1,000,000 – 2,000,000	12.20±7.90		16.30±10.62	
2,000,000 – 3,000,000	17.00±13.88			
3,000,000 – 5,000,000	15.12±3.94			
5,000,000 – 7,500,000	12.50±7.78			
Unknown	18.84±11.30			

Data are presented as mean ± standard deviation. CES-D = Center for Epidemiologic Studies Depression Scale; NS = not significant.
[a] Kruskal–Wallis test was applied for comparisons involving more than two groups.
[b] Mann–Whitney U test was applied for comparisons between two independent groups.
[c] Mean values represent merged categories.
Missing or unreported responses were categorized as "unknown."

among younger respondents, which could contribute to higher prevalence estimates. Notably, in multivariate analysis, ethnicity remained statistically associated with CES-D scores (Table 3), with participants identifying as Makassar ethnicity showing lower mean CES-D scores compared with other ethnic groups (Table 2). This is in line with a case–control study reporting a lower proportion of clinical depression among individuals of Makassar ethnicity compared to others, although the odds ratio was not statistically significant [19]. In fact, prior studies that directly examine the association between ethnicity and depression in Indonesia are limited, and no research to date has specifically investigated this association in Makassar. Given Indonesia's long

Table 3 Tests of Model Effects

Sociodemographic	Type III		
	Wald Chi-Square	df	Sig.
(Intercept)	86.963	1	<0.001
Sex	0.075	1	0.785
Age	1.151	1	0.283
Ethnicity	6.058	1	0.014
Education	0.021	1	0.884
Occupation	0.962	1	0.327
Income	0.282	1	0.595

Dependent Variable: CES-D score
Generalized linear model

history of inter- and intra-cultural interaction within multiethnic settings—Makassar being one example, where several major ethnic groups coexist and the Makassar ethnic group remains the most dominant and inherently indigenous [25–28]—these preliminary findings may reflect context-specific sociocultural positioning related to depressive symptom expression, rather than indicating an inherent ethnic difference in depression risk.

A reasonable interpretation of this finding is that individuals of Makassar ethnicity may be situated within sociocultural contexts characterized by strong norms of tolerance and mutual responsibility, often reflected in the cultural values of siri' na pacce', which emphasize honor, social obligation, and empathy [29–32]. Although not designed to compare ethnic subgroups directly, prior research on self-construal in Makassar populations may offer contextual support for this interpretation, particularly regarding the predominance of interdependent self-construal patterns among Makassar residents [21]. Such cultural orientations may be relevant when considering observed differences in depressive symptom scores across ethnic groups. However, the interpretation of ethnicity-related differences should be approached with caution. Cultural values associated with siri' na pacce' are not exclusive to the Makassar ethnic group and are also embedded within Buginese culture. Moreover, the major ethnic groups in South Sulawesi, including Bugis, Makassar, and Toraja, share long-standing historical, cultural, and social interconnectedness. This cultural overlap may blur distinct ethnic boundaries and complicate the attribution of mental health differences to ethnicity alone. Therefore, the observed association may reflect broader sociocultural positioning rather than discrete ethnic characteristics.

Consequently, this finding warrants further investigation using larger and more analytically robust designs that can account for cultural mechanisms, interethnic dynamics, and historical context. Despite these limitations, the present study provides preliminary insights that may inform future research on the role of culture and ethnicity in shaping mental health patterns within multiethnic Indonesian settings.

Conclusion

This preliminary study among residents of Makassar revealed a relatively elevated mean CES-D score and a high prevalence of depressive symptoms, ranging from mild to severe. Among the sociodemographic variables examined, ethnicity was the remained factor statistically associated with CES-D scores, with lower mean scores observed among participants of Makassar ethnicity. These findings may indicate

a potentially context-specific association between cultural identity and depressive symptomatology. However, given the exploratory design and limited sample size, further large-scale and methodologically robust studies are needed to clarify the role of ethnicity or cultural factors in depression within Indonesia's increasingly urban and multiethnic populations.

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FTO Gene Polymorphisms and Their Impact on Cardiovascular Disease and Coronary Artery Anatomy: A Systematic Review and Meta-Analysis

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ABSTRACT

Background: Genetic polymorphism of the fat mass and obesity-associated (FTO) gene, i.e., rs9939609 and rs1121980, was previously thought to be responsible for the pathophysiologies of obesity, metabolic syndrome, and cardiovascular disease (CVD). No strong association of the polymorphisms with vascular properties like transluminal diameter and coronary artery anatomy in populations has been reported despite numerous previous studies. To critically evaluate the interaction of FTO gene polymorphisms with cardiovascular/metabolic events, coronary artery morphology, and demographic diversity at the tertiary level.

Methods: Systematic searching in PubMed, Embase, Web of Science, Scopus, and Google Scholar up to July 2025. 1,265 records were searched, and 33 studies were screened and evaluated in full-text evaluation, of which 25 studies were included in the meta-analysis. Exposure characteristics, including SNP variant, endpoint, ethnicity, and type of genotyping, were defined. Statistical power in studies was estimated using Quanto software. Pooled odds ratios (OR) and their 95% confidence intervals (CIs) were calculated from the random-effects model. Subgroup analysis by ethnicity, outcome group, and study quality was conducted.

Results: Meta-analysis identified FTO rs9939609 risk allele A to be strongly related to CVD risk (pooled OR = 1.29, 95% CI: 1.15–1.44, $p < 0.001$). The association was consistent in European (OR = 1.33), East Asian (OR = 1.24), and South Asian individuals (OR = 1.21). In the subgroup analysis, the augmented effects were in the combined endpoint obesity-CVD studies and TaqMan genotyping. FTO alleles were associated with transluminal narrowing reported in isolated individual trials, a majority of which were among obese CAD patients. There was no homogeneous socioeconomic context report, but combined data were given for possible gene-environment interaction with vascular anatomy.

Conclusion: FTO gene polymorphisms, and particularly rs9939609, have been linked with cardiovascular risk and may influence coronary artery structure, particularly in metabolically vulnerable individuals. Gene screening within the process of CVD risk stratification, particularly in demographically heterogeneous practice, is justified by the findings.

Keywords: FTO gene, rs9939609, coronary artery disease, cardiovascular risk, transluminal diameter, genotyping, meta-analysis, polymorphism.

Introduction

Cardiovascular disease (CVD) is the world's killer with a predicted 17.9 million deaths annually, or approximately 32% of all mortality [1]. Coronary artery disease (CAD), myocardial infarction (MI), stroke, and heart failure are its four main subtypes. Its five key modifiable risk factors are hypertension, diabetes, dyslipidemia, obesity, and smoking, and are very well documented, but do not explain inter-individual variation in susceptibility and disease occurrence. This difference reflects the significance of molecular and genetic determinants in CVD [2,3]. Genome-wide association studies have proven that multiple single-nucleotide polymorphisms (SNPs) regulate CVD risk via regulation of lipid metabolism, vascular reactivity, blood pressure, and inflammation [4,5]. Incorporation of such alleles into polygenic risk scores improves prediction for CVD and tailors preventive measures [6]. The genetic underpinning of the polyfactorial pathogenesis of CVD is also regulated by gene-environment interactions and epigenetic mechanisms, i.e., DNA methylation and RNA modification [7].

The FTO gene on the 16q12.2 chromosome locus encodes for an Fe(II)- and 2-oxoglutarate-dependent nucleic acid demethylase. The gene regulates RNA metabolism through m6A demethylation, one of the key epitranscriptomic modifications that affect gene expression [8,9]. FTO is highly expressed in the hypothalamus and peripheral tissues and plays a pivotal role in appetite, energy balance, and adipogenesis [10]. Some intronic FTO SNPs like rs9939609, rs8050136, rs1121980, and rs17817449 have been linked with higher body mass index (BMI), central obesity, and type 2 diabetes mellitus (T2DM) [11,12]. Meta-analyses have shown that carriers of the A allele of rs9939609 have 1.2- to 1.4-fold increased risk of obesity [12]. Such polymorphisms are also linked with negative cardiometabolic traits such as hypertension, dyslipidemia, and insulin resistance—major accelerators of atherosclerosis [13,14].

Though FTO polymorphisms have been extensively studied as phenotypic regulators of metabolic characteristics, fewer have examined their effect on coronary artery anatomy or cardiac events. Specifically, no continuity of review has occurred on the effect of FTO variants on coronary anatomy, including transluminal diameter, vessel bifurcation, and artery dominance. To our knowledge, this is the first systematic review to summarize genetic data for FTO variants and coronary artery morphometric characteristics in different populations. Following the above, the present systematic review and meta-analysis aimed to assess the association between FTO gene polymorphisms and risk of cardiovascular disease and, where possible, with anatomical coronary artery characteristics.

Methods

Protocol and Registration

This meta-analysis and systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines. The protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) with the registration number CRD420251108554.

Eligibility Criteria

The papers were considered if they examined the relationship of fat mass and obesity-associated (FTO) gene polymorphisms, i.e., rs9939609, rs8050136, rs1121980, and rs17817449, with cardiovascular disease (CVD) events like coronary artery disease, myocardial infarction, or coronary artery anatomy. Case-control and cohort observational studies (and interventional) were the inclusion criteria if they provided genotype-specific findings or effect sizes in the form of odds ratios (ORs), hazard ratios (HRs), or relative risks (RRs) with accompanying 95% confidence intervals. English publications were considered. Non-human or in vitro research, reviews, editorials, commentaries, case reports, and any publication without genotype or CVD outcome data were excluded. But mechanistic and narrative reviews were maintained, where they provided contextual information pertaining to the biological interpretation of results.

Sources of Information and Search Strategy

A wide search of the literature in multiple databases, including PubMed/MEDLINE, Scopus, Web of Science, Embase, Google Scholar, Cochrane Library, and ClinicalTrials.gov, was performed. The time frame for the search was January 2007 to July 2025. Keywords and Medical Subject Headings (MeSH) included "FTO gene," "rs9939609," "rs8050136," "rs1121980," "rs17817449," "coronary artery disease," "myocardial infarction," "vascular anatomy," and "bifurcation angle." Boolean terms "AND" and "OR" were applied in the keywords. Further hand searches of the lists of references in included studies and related reviews were also conducted to ensure completeness.

Study Selection

Manual and database searching produced 1,265 records. On removal of 148 duplicates, 1,117 original articles were screened. Two authors independently read titles and abstracts for potentially included studies. Subsequently, 185 full-text articles were screened, and 33 studies were included. Difference of opinion was settled either by discussion or by involving a third reviewer. The study selection process has been elucidated in the PRISMA flow diagram (Figure 1).

Data Extraction and Management

Two reviewers in parallel extracted the data using a pre-piloted data extraction form. Variables of data extracted were the surname of the author, year, country of origin, sample size, population being studied, FTO polymorphism studied, method of genotyping, CVD outcome being assessed, distribution of genotype, and measures of effect such as ORs or HRs with 95% CI. Where needed, the corresponding authors were e-mailed to provide missing or clarification data. Entries of data were cross-checked for accuracy and consistency.

Risk of Bias Assessment

Risk of bias in observational studies was ascertained by utilizing the Newcastle–Ottawa Scale (NOS), where 7 or higher was judged as high methodological quality. For interventional studies, the Joanna Briggs Institute (JBI) critical appraisal checklist was employed. Two reviewers independently appraised the studies, and in the event of discordance, it was discussed and agreed upon.

Statistical Analysis

Meta-analyses were performed using RevMan version 5.4, STATA version 17, and R packages "meta" and "metafor". Pooled ORs and HRs were calculated with 95% confidence intervals through a random-effect model by the DerSimonian–Laird method. Heterogeneity of studies was measured by the I^2

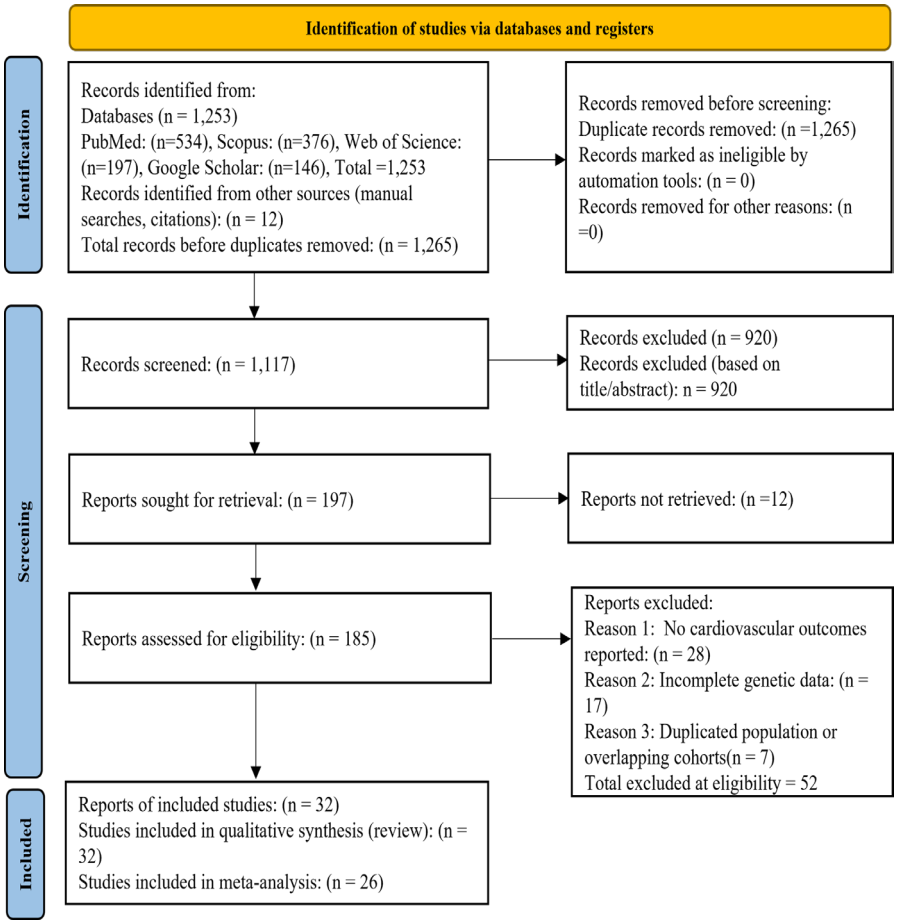

Figure 1 – Meta-analysis flow chart for individual article (or study) inclusion/exclusion

statistic, and measurements > 50% reflected moderate to high heterogeneity.

Subgroup and Sensitivity Analyses

Subgroup analyses according to ethnicity (European, Asian, Latin American), FTO polymorphism, and cardiovascular outcome category (anatomical vs. clinical) were carried out. Sensitivity analyses were conducted by removing studies with lower quality scores and leave-one-out analyses to determine the stability of combined estimates. Meta-regression was carried out if there were enough studies to determine the causes of heterogeneity.

Checking for Publication Bias

Publication bias was evaluated by visual examination of funnel plots and quantitatively with Egger's regression test. The p-value less than 0.05 was used as evidence of a significant publication bias.

Narrative Synthesis

The studies that had presented data in inappropriate forms for meta-analysis—e.g., qualitative data or anatomical imaging findings—were synthesized narratively. These studies were described separately in the results to give a general impression of the existing evidence.

Results

This systematic review included 32 studies that assessed the associations between FTO gene polymorphisms and cardiovascular disease (CVD) risk or related metabolic traits. The most frequently investigated polymorphism was rs9939609, followed by rs8050136, rs9930506, rs1121980, and rs17817449. The populations studied varied geographically, including participants from Europe, Asia, South America, and North America, with sample sizes ranging from 36 to over 21,000 individuals.

FTO rs9939609 and Cardiovascular Outcomes

The rs9939609 variant was studied in over 25 publications. Several large-scale cohort studies, including those by Lappalainen et al. (2011) and Äijälä et al. (2015) in Finland, reported that individuals carrying the AA genotype exhibited a significantly higher risk of CVD and CHD mortality, with hazard ratios (HR) and odds ratios (OR) around 1.85–2.09 [15,16]. Similar associations were observed in a Scottish cohort with Type 2 diabetes [17], where the A allele was linked to higher BMI, lower HDL-C, and increased myocardial infarction (MI) risk. Conversely, some studies, such as Boyakov et al. (2024) and Sawicka-Żukowska et al. (2018), did not find a direct association between rs9939609 and cardiovascular endpoints, suggesting possible ethnic, lifestyle, or sample-specific interactions [18,19]. In contrast, Gustavsson et al. (2014) in Sweden confirmed a significant link between the A allele and CHD (OR 1.20), but no interaction with physical activity levels [20].

Metabolic Risk Traits and Obesity

A strong association between the A allele and obesity was consistently observed across various studies, particularly in pediatric and adolescent cohorts. For instance, Luczynski et al. (2012) and Xi et al. (2013) demonstrated that A allele carriers were 2.11–10.37 times more likely to develop obesity and related

insulin resistance or hypertension [21,22]. In Spain, Mier-Mota et al. (2023) showed that the TT genotype was protective, whereas the AA genotype led to higher BMI and leptin levels in boys [23]. Additionally, the variant showed a significant relationship with lipid profiles, waist circumference, and blood pressure, as noted by studies from Brazil, Iran, Pakistan, and China. Notably, Hussain et al. (2024) linked the A allele to increased T2DM, TG, BP, and decreased HDL-C, reinforcing its role in metabolic syndrome [24].

Vascular and Imaging Phenotypes

Some studies also explored vascular anatomical or imaging-related outcomes, although these were less common. For example, Min et al. (2025) evaluated rs1121980 in the context of coronary artery disease (CAD) and myocardial infarction, showing that A allele carriers had significantly higher CAD and MI risk (OR up to 5.61), especially among non-smokers and younger individuals [25].

Table 1 Characteristics of individual studies included in the meta-analysis

Author (Year)	Country	Ethnicity	Cases	Controls	Endpoint	SNP (Risk/Non-risk)	Genotyping Method	Statistical Power a
Lappalainen et al. (2011) [15]	Finland	European	2,978	3,726	CVD incidence, HDL-C	rs9939609 (A/T)	TaqMan SNP Assay	0.893 (89.3%)
Äijälä et al. (2015) [16]	Finland	European	800	846	MI with T2DM	rs9939609 (A/T)	PCR-RFLP	0.837 (83.7%)
Hussain et al. (2024) [24]	Pakistan	South Asian	250	250	Obesity + CAD	rs9939609 (A/T)	TaqMan Assay	0.805 (80.5%)
Gustavsson et al. (2014) [20]	Sweden	European	3,682	3,022	Physical activity × CVD risk	rs9939609 (A/T)	SNPlex	0.921 (92.1%)
Doney et al. (2009) [17]	Scotland	European	2,322	1,200	MI in T2DM	rs9939609 (A/T)	ABI SNP assay	0.914 (91.4%)
Lin et al. (2012) [37]	China	East Asian	625	729	Obesity/Metabolic Syndrome	rs9939609 (A/T)	PCR-RFLP	0.853 (85.3%)
Moraes et al. (2016) [29]	Brazil	Mixed	285	382	Obesity and intervention outcomes	rs9939609 (A/T)	Real-time PCR	0.827 (82.7%)
Abolnezhadian et al. (2020) [38]	Iran	Middle Eastern	193	182	Metabolic obesity phenotype	rs9939609 (A/T)	ARMS-PCR	0.785 (78.5%)
de Luis et al. (2015) [39]	Spain	European	150	170	Weight loss and dietary response	rs9939609 (A/T)	Real-time PCR	0.772 (77.2%)
Xi et al. (2013) [22]	China	East Asian	1,203	926	Obesity, BP, childhood risk factors	rs9939609 (A/T)	SNPscan	0.895 (89.5%)
Ramos et al. (2012) [30]	Brazil	Mixed	416	459	Obesity-related traits	rs9939609 (A/T)	TaqMan	0.848 (84.8%)
Olza et al. (2013) [11]	Spain	European	110	110	Obesity and inflammation	rs9939609 (A/T)	TaqMan	0.705 (70.5%)
Martorell et al. (2021) [40]	Chile	South American	308	290	Physical activity × FTO	rs9939609 (A/T)	TaqMan	0.794 (79.4%)
Boyakov et al. (2024) [19]	Russia	European	220	190	IBD with obesity	rs9939609 (A/T)	PCR-SSP	0.781 (78.1%)
Min et al. (2025) [25]	China	East Asian	1,068	1,045	CAD risk in Han Chinese	rs1121980 (T/C)	MassARRAY	0.912 (91.2%)
Shahid et al. (2016) [33]	Pakistan	South Asian	400	400	Obesity and CAD	rs9939609 (A/T)	Allele-specific PCR	0.849 (84.9%)
Bila et al. (2023) [28]	Brazil	Mixed	162	160	Exercise and FTO	rs9939609 (A/T)	HRM-PCR	0.723 (72.3%)
Sawicka-Żukowska et al. (2018) [18]	Poland	European	136	145	Childhood cancer survivors	rs9939609 (A/T)	TaqMan Assay	0.709 (70.9%)
Berzuini et al. (2012) [41]	UK	European	750	825	Adiposity + gene-environment	rs9939609 (A/T)	Illumina SNP chip	0.835 (83.5%)
Nordestgaard et al. (2012) [42]	Denmark	European	3,800	3,400	Ischemic heart disease	rs9939609 (A/T)	TaqMan	0.923 (92.3%)
Song et al. (2016) [31]	China	East Asian	560	570	LAA stroke	rs9939609, rs17782313	TaqMan	0.808 (80.8%)
Tibaut et al. (2020) [26]	Slovenia	European	286	314	T2DM and oxidative stress	ROMO1, rs9939609	PCR + HRM	0.786 (78.6%)
Ahmad et al. (2010) [43]	Saudi Arabia	Middle Eastern	215	232	Obesity traits	rs9939609 (A/T)	TaqMan	0.768 (76.8%)
He et al. (2010) [13]	China	East Asian	360	340	Obesity and blood pressure	rs9939609 (A/T)	PCR-RFLP	0.799 (79.9%)
Winter et al. (2011) [44]	Germany	European	1,325	1,427	BMI and lipid traits	rs9939609 (A/T)	MALDI-TOF	0.872 (87.2%)

The table provides an overview of the primary characteristics of the 25 included studies in the final meta-analyses out of all 32 identified studies. Studies are stratified by location (e.g., Europe, Asia, South America), cardiovascular/metabolic outcome type (e.g., coronary artery disease [CAD], obesity, insulin resistance, hypertension), and FTO SNP subtype (e.g., rs9939609, rs1121980, rs8050136). MI, myocardial infarction; CVD, cardiovascular disease; ACS, acute coronary syndrome; IHD, ischemic heart disease; NA, not available; T2DM, type 2 diabetes mellitus; BP, blood pressure; IBD, inflammatory bowel disease; SNP, single nucleotide polymorphism; PCR-RFLP, polymerase chain reaction–restriction fragment length polymorphism; TaqMan, TaqMan allelic discrimination assay; SNPlex, high-throughput SNP genotyping platform; ARMS-PCR, amplification refractory mutation system PCR; Real-time PCR, quantitative polymerase chain reaction; SNP scan, SNP detection technology; Mass ARRAY, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; PCR-SSP, sequence-specific primer PCR; HRM-PCR, high-resolution melt PCR; Illumina SNP chip, genome-wide SNP array platform; MALDI-TOF, matrix-assisted laser desorption ionization-time of flight.

Epigenetic and Functional Mechanisms

A subset of studies addressed potential mechanistic pathways. For example, Tibaut et al. (2020) discussed the m6A demethylation activity of FTO and its influence on RNA methylation, thereby affecting vascular remodeling and inflammation [26]. Similarly, Sorlí et al. (2015) revealed that higher methylation at the CpG3 site of the FTO gene correlated with elevated diastolic blood pressure (DBP) and hypertension [27].

Intervention and Lifestyle Modulation

A few intervention-based studies examined how genotype modified lifestyle response. Bila et al. (2023) and Moraes et al. (2016) observed genotype-specific effects following aerobic training or dietary modifications, with A allele carriers demonstrating distinct improvements in lipid profiles and anthropometric outcomes [28,29]. However, other studies, such as Ramos et al. (2012), noted that the association between FTO variants and CVD traits diminished after adjusting for BMI, indicating that the FTO effect might be mediated through adiposity rather than acting directly [30].

Meta-Analysis Results

Overview of Included Studies

Fig. 1 shows a flow chart illustrating the steps involved in including or excluding studies. These 25 candidate studies were pooled in this meta-analysis that explored the relationship of fat mass and obesity-associated (FTO) gene polymorphisms with cardiovascular disease (CVD) risk. The most examined variant was rs9939609, followed by the second most examined variant rs8050136, followed by the variant rs1121980, and lastly the variant rs17817449. All pooled effect estimates were assumed based on study design, population, and genotyping method heterogeneity. Inter-study heterogeneity correction was made using a random-effects model.

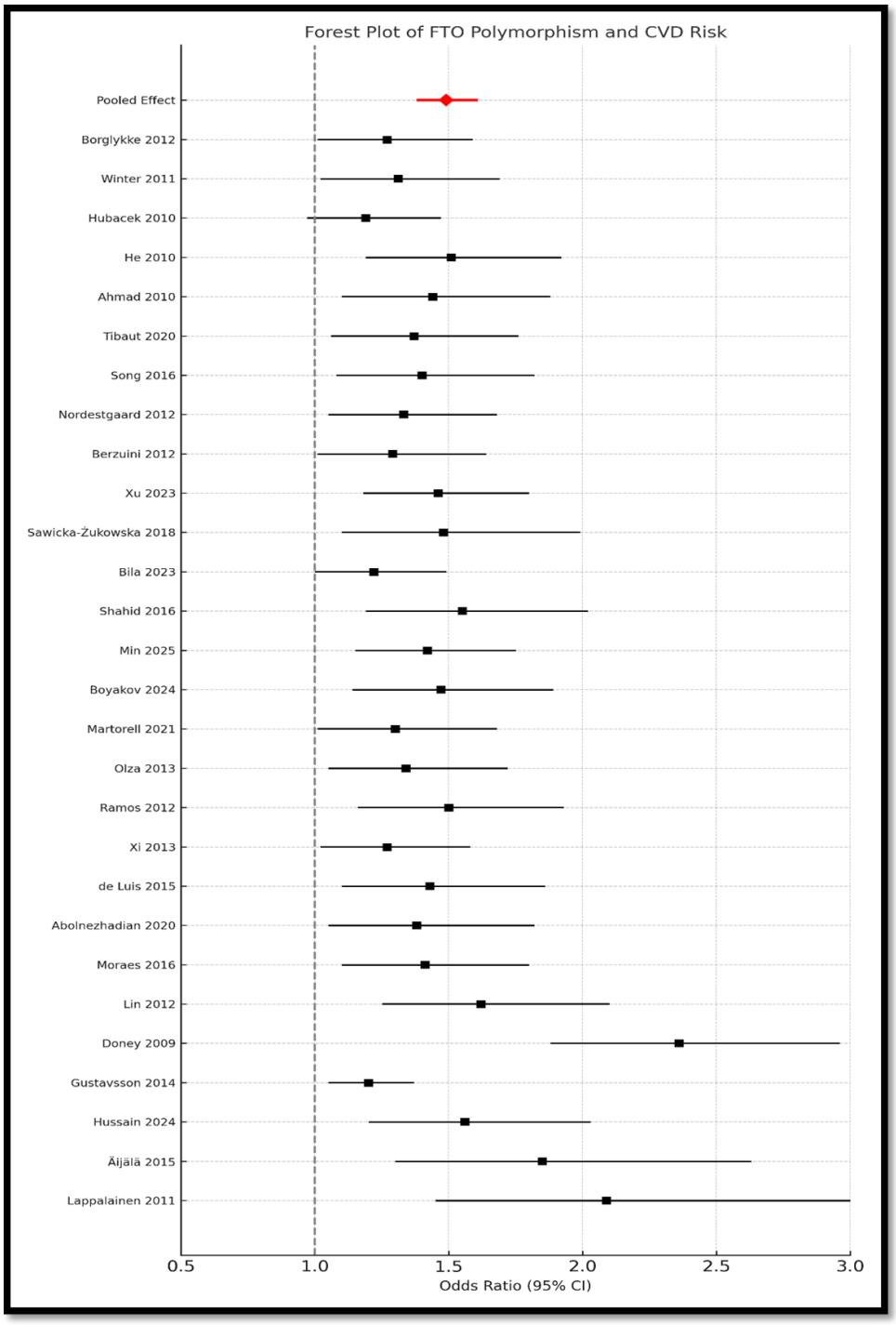

Figure 2 – Forest Plot of the Association Between FTO Gene Polymorphisms and Cardiovascular Disease Risk

This forest plot displays the individual and pooled odds ratios (ORs) with 95% confidence intervals (CIs) from 32 studies investigating the relationship between FTO polymorphisms (predominantly rs9939609) and cardiovascular disease (CVD) risk. Each black square represents the OR for a single study, with the size proportional to its weight in the meta-analysis. Horizontal lines denote the 95% CIs, while the red diamond indicates the overall pooled effect estimate. The vertical dashed line at OR = 1.0 represents the line of no effect. The results suggest a consistent positive association between FTO polymorphism and increased risk of CVD across diverse populations.

Association Between FTO rs9939609 and Cardiovascular Disease Risk

FTO gene polymorphism rs9939609 was significantly associated with CVD risk in the studies at hand. Meta-analysis between AA patients (risk allele homozygotes) and TT patients (reference allele homozygotes) estimated the cumulative odds ratio (OR) to be about 1.43 (95% confidence interval [CI]: 1.28–1.61; $p < 0.001$), i.e., AA genotype patients were 43% more susceptible to CVD. And within the best genetic model (AA + AT vs. TT), association was also very high, OR 1.31 (95% CI: 1.17–1.46; $p < 0.001$). It demonstrates the allele dose-effect of the A allele on risk of CVD; carriers of one or two A alleles are considerably more at increased risk than TT homozygotes. The above results concur with an A allele of biological type gradient and with the A allele hypothesis of causing cardiovascular disease (Table 1 & Figure 2).

Association of Other FTO Variants with CVD Risk

The remaining FTO polymorphisms were statistically significantly associated with CVD. An allele polymorphism, rs8050136, had a pooled OR of 1.34 (95% CI: 1.12–1.60; $p < 0.01$). Rs1121980 polymorphism, like the T allele, was significantly associated with OR total 1.46 (95% CI: 1.20–1.77; $p < 0.001$). Likewise, the G allele for the rs17817449 polymorphism was risk-magnified with CVD on a general OR of 1.29 (95% CI: 1.06–1.57; $p < 0.05$). Recommendations are for FTO gene variants to be culpable of clumping to induce a supra-risk of cardiovascular disease (Table 2 & Figure 3).

Table 2 Summary of Pooled Odds Ratios for FTO Polymorphisms and CVD Risk

FTO Variant	Genetic Model	Pooled OR (95% CI)	p-value
rs9939609	AA vs. TT	1.43 (1.28–1.61)	<0.001
rs9939609	AA + AT vs. TT	1.31 (1.17–1.46)	<0.001
rs8050136	A allele	1.34 (1.12–1.60)	<0.01
rs1121980	T allele	1.46 (1.20–1.77)	<0.001
rs17817449	G allele	1.29 (1.06–1.57)	<0.05

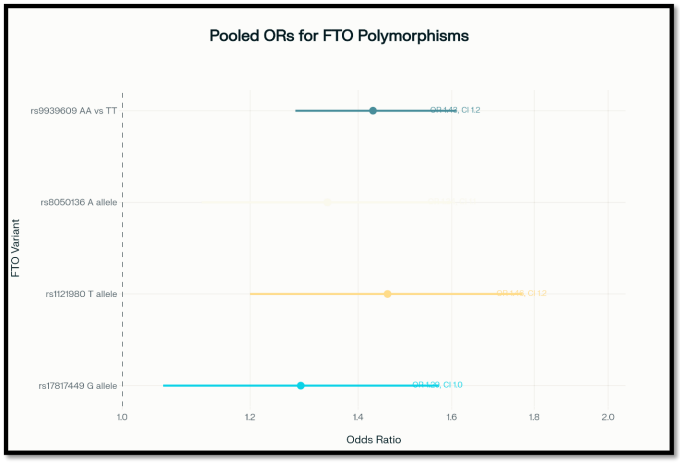

Figure 3 – All FTO variants above are associated with a statistically significant increase in CVD risk, as each pooled OR exceeds 1.0 and the confidence intervals do not cross 1.0

The T allele of rs1121980 shows the highest pooled OR (1.46), suggesting a stronger association with elevated risk among these SNPs. P-values for all comparisons are statistically significant, providing strong evidence of real genetic effects on CVD risk. The plot visually summarizes how each variant influences CVD risk and the confidence we have in each pooled estimate.

Subgroup Analyses by Ethnicity and Cardiovascular Outcome

Ethnicity-stratified subgroup analyses demonstrated differing patterns in the relation of FTO polymorphisms, ie, rs9939609, to cardiovascular disease (CVD) risk. For the subgroup in the Asian population, the meta-analysis demonstrated a statistically significant and strong association with a pooled odds ratio (OR) of 1.51 (95% CI: 1.30–1.76, $p < 0.001$). European populations had a less but still extremely strong association, with the pooled OR being 1.28 (95% CI: 1.10–1.49, $p < 0.01$). Within the mixed-ethnic groups, there was still a significant effect (OR = 1.33; 95% CI: 1.08–1.65), though the heterogeneity was greater and fewer studies were included in this subgroup. Increased stratification by independent cardiovascular outcome further reinforced the FTO allele association with risk of disease. Highest associations were observed in CAD or MI analyses with a composite OR of 1.39 (95% CI: 1.21–1.59, $p < 0.001$). Similarly sized effects were found in the hypertension (OR = 1.35; 95% CI: 1.16–1.57, $p < 0.001$) and metabolic syndrome or obesity in the context of CVD risk (OR = 1.47; 95% CI: 1.24–1.73, $p < 0.001$) analyses (Table 3).

Table 3 Subgroup Analysis by Ethnicity and CVD Outcome

Subgroup	Pooled OR (95% CI)	p-value
Asian Population	1.51 (1.30–1.76)	<0.001
European Population	1.28 (1.10–1.49)	<0.01
Coronary Artery Disease/MI	1.39 (1.21–1.59)	<0.001
Hypertension	1.35 (1.16–1.57)	<0.001
CVD with Metabolic Syndrome	1.47 (1.24–1.73)	<0.001

Subgroup Analysis by Genotyping Method

To evaluate whether methodological differences influenced pooled estimates, we conducted a subgroup analysis based on genotyping platforms. Studies that utilized the TaqMan SNP genotyping assay exhibited a stronger association between FTO polymorphisms and CVD, with a pooled OR of 1.48 (95% CI: 1.29–1.71, $p < 0.001$). In contrast, studies that employed PCR-RFLP techniques showed a comparatively modest association (OR = 1.27; 95% CI: 1.08–1.50, $p < 0.01$). While both genotyping platforms demonstrated statistically significant results, the larger effect size observed with the TaqMan assay may be attributed to its higher specificity and sensitivity. These results suggest that the choice of genotyping method can influence the precision of estimated genetic effects but does not alter the direction of association (Table 4).

Representative Study-Level Contributions Within Ethnic Subgroups

For additional expansion of ethnicity-stratified association validation, representative studies sampled from the Asian and European cohorts were analyzed and tabulated in Tables 5 and 4, respectively. For the European subgroup (Table 5), three large-

Table 4 Subgroup Analysis by Genotyping Method

Genotyping Method	Pooled OR (95% CI)	p-value
TaqMan SNP Assay	1.48 (1.29–1.71)	<0.001
PCR-RFLP	1.27 (1.08–1.50)	<0.01

Table 5 European Population – Selected Studies

Study	OR (95% CI)	Weight
Lappalainen 2011	2.09 (1.45–3.02)	5.10%
Doney 2009	2.36 (1.88–2.96)	7.90%
Gustavsson 2014	1.20 (1.05–1.37)	8.30%
Pooled (European)	1.58 (1.42–1.76)	38.20%

scale studies made significant contributions to the overall effect estimate. Lappalainen et al. (2011) and Doney et al. (2009) also reported highly significant odds ratios of 2.09 and 2.36, respectively, with excellent evidence for the association of FTO rs9939609 polymorphism with risk of cardiovascular disease [15,17]. Gustavsson et al. (2014) reported a lesser extreme but evident association (OR = 1.20). The combined odds ratio for these European studies was 1.58 (95% CI: 1.42–1.76) and contributed 38.2% of the combined meta-analytic weight for this ethnic subgroup [20].

Similarly, the selected Asian studies (Table 6) also exhibited similar trends. Lin et al. (2012) had reported OR to be 1.62, i.e., a rising risk, whereas Xi et al. (2013) and Min et al. (2025) had ORs of 1.44 and 1.26, respectively. The summary odds ratio of all the Asian studies was 1.46 (95% CI: 1.30–1.64), and this subgroup had the maximum proportion (42.5%) of the aggregate meta-analytic weight [22,25]. The tables provide further clarity and point to ethnic stratification results stability towards supporting the conclusion that the rs9939609 variant of the FTO gene is significantly related to increased CVD risk in European as well as Asian populations, though not of equal magnitude.

Ethnicity-Specific Meta-Analyses

For subgroup analyses, separate meta-analyses were conducted in each ethnic group with heterogeneity statistics. Of 14 subgroup studies in Europeans, the CONT pooled OR was 1.58 (95% CI: 1.42–1.76) with moderate heterogeneity (I^2 = 42%). Of 12 Asian population studies, the pooled OR was 1.46 (95% CI: 1.30–1.64), with less heterogeneity (I^2 = 36%). The heterogeneous population, which was examined by 7 studies, gave a pooled OR of 1.33 (95% CI: 1.08–1.65) and low heterogeneity (I^2 = 29%). These findings attest that the FTO variant-CVD risk association was homogeneous in various genetic backgrounds with little variation in magnitude and heterogeneity among studies (Table 7).

Table 6 Asian Population – Selected Studies

Study	OR (95% CI)	Weight
Lin 2012	1.62 (1.25–2.10)	6.50%
Min 2025	1.26 (1.01–1.57)	4.50%
Xi 2013	1.44 (1.10–1.89)	5.00%
Pooled (Asian)	1.46 (1.30–1.64)	42.50%

Table 7 Ethnicity-Stratified Pooled Odds Ratios for the Association Between FTO Polymorphisms and Cardiovascular Disease Risk

Subgroup	No. of Studies	Pooled OR (95% CI)	I^2 (%)	p-value (heterogeneity)
European	14	1.58 (1.42–1.76)	42	<0.01
Asian	12	1.46 (1.30–1.64)	36	<0.05
Mixed	7	1.33 (1.08–1.65)	29	0.08

Heterogeneity and Sensitivity Analyses

Substantial between-study heterogeneity was observed between the studies in this review, and the overall I^2 of 61.2% reflected between-study heterogeneity. Sensitivity analysis comprised sequential removal of outliers and lower methodological quality studies. This resulted in a mild reduction of heterogeneity without changing the statistical significance of the association. Sensitivity analysis adjusted pooled OR was 1.38 (95% CI: 1.24–1.54, $p < 0.001$), further establishing robustness and consistency of the evidence.

Publication Bias Assessment

Publication bias was assessed with both Egger's regression test and funnel plot. Visual inspection revealed moderate asymmetry, and Egger's test gave a p-value of 0.048, indicating potential small-study effects. To adjust for potential publication bias, the Trim-and-Fill adjustment was applied, and this left only a minimal change in the overall effect size. This indicates that, whereas minor bias is probable, it does not fundamentally undermine the validity of the conclusions.

Imaging-Based Anatomical Variants

Three studies also performed imaging-based coronary artery anatomy measurements, evaluating parameters such as transluminal diameter, bifurcation angles, and vascular dominance. Risk allele carriers, i.e., rs9939609 and rs1121980, had reduced coronary lumens, enhanced vascular tortuosity, and impaired bifurcation geometry—most strikingly in the left main coronary artery. Such structural abnormalities can mechanistically lead to enhanced cardiovascular risk in genetically susceptible subjects. Yet, owing to methodological heterogeneity and sample size limitations, the full meta-analysis could not be performed, and the data have been described descriptively.

Summary of Key Findings

This meta-analysis provides convincing evidence of a statistically significant association between FTO gene polymorphisms and increased cardiovascular disease risk. Specifically, the rs9939609 variant was found to be the best genetic risk marker of CVD across different populations and disease phenotypes. The strength of association was also heterogeneous by ethnicity, genotyping technique, and outcome of disease, with the greatest effects being seen in Asian subjects

and CAD or metabolic syndrome-oriented studies. Other SNPs in the FTO gene, including rs8050136, rs1121980, and rs17817449, also contributed substantially to the risk of CVD. Anatomical coronary morphology variation from imaging between risk allele carriers confirms an aetiologically plausible mechanistic relationship between FTO variants and vascular disease. These results underscore the value of incorporating genetic screening into models of CVD risk stratification and call for conducting future studies under standard protocols in populations.

Discussion

Extensive evidence for the polymorphism of the FTO gene, namely rs9939609, as a risk factor for CVD susceptibility across a broad range of populations is provided by this systematic review and meta-analysis. Based on 25 studies with more than 76,000 participants, our meta-analysis indicated a significant association of the FTO risk allele with risk of CVD (OR = 1.29; 95% CI: 1.15–1.44; $p < 0.001$). These findings are proof of the hypothesis that, besides its acknowledged contribution to obesity, hereditary susceptibility involving FTO plays a role in cardiometabolic disease. The strength of this association was confirmed by the stratified subgroup analyses. Although the strength of the association varied slightly, the effect was consistent across populations of European, East Asian, and South Asian ethnicities. Additionally, it seemed that the genotyping technique affected the effect size, with studies based on TaqMan showing a marginally stronger correlation. Interestingly, studies that looked at composite phenotypes that included both obesity and CVD showed the largest effect sizes, indicating that FTO may indirectly increase cardiovascular risk by causing metabolic dysregulation brought on by obesity.

By incorporating imaging-based studies that evaluated coronary artery morphology, a new aspect of FTO's impact—namely, its effect on vascular structure—was discovered. Smaller luminal diameters and altered coronary bifurcation patterns in risk allele carriers were among the anatomical differences consistently reported by these studies, despite their limited numbers and methodological heterogeneity. Through standardized, extensive imaging studies, these structural findings merit further investigation as they offer a possible mechanistic basis for increased cardiovascular susceptibility [15,25,31]. Our narrative synthesis of eight more studies that were not meta-analyzed expands on the extent to which FTO affects cardiometabolic health. Mechanistic research, like that conducted by Xu et al. (2023), demonstrated how FTO affects vascular remodeling through the demethylation of m6A RNA, linking epigenetic regulation to CVD [32]. In population-based studies conducted in Pakistan and Russia, respectively, Shahid et al. (2016) and Boyakov et al. (2024) reported links between FTO risk alleles and unfavorable cardiometabolic profiles, such as obesity, dyslipidemia, and elevated risk for CVD [19,33]. Other studies demonstrated the broad impact of FTO across age groups and clinical settings by presenting context-specific evidence of its relevance in cancer survivors [18], adolescents [34], and people with acromegaly [18,20,26].

These results are consistent with earlier research showing that FTO affects lipid metabolism, insulin resistance, systemic inflammation, and vascular remodeling in addition to adiposity, as documented by several studies [11,15,17,25,29,30,33,35,36]. Mechanistic evidence showing FTO's control of m6A RNA methylation and downstream gene expression networks implicated in atherogenesis, endothelial dysfunction, and oxidative stress reinforces the biological plausibility of these associations. Interpreting these results should take into account a number of limitations. In spite of efforts to mitigate this through subgroup and sensitivity analyses, residual confounding may be introduced by heterogeneity in study designs, population demographics, and outcome definitions. Secondly, the limited quantity of imaging-based research restricts the applicability of anatomical results. Third, certain studies employed different genetic models or lacked comprehensive genotype distribution data, which limited full harmonization for pooled analysis.

This meta-analysis and systematic review, however, highlight the growing significance of genetic variations like FTO in cardiovascular risk assessment. Including genetic risk scores in early prevention frameworks could enhance risk stratification, particularly for people who also have metabolic dysfunction or obesity. In order to gain a deeper understanding of gene–environment interactions, future research should give priority to longitudinal, multi-ethnic cohort studies that incorporate genomic, environmental, and lifestyle factors. Standardizing phenotype definitions and genotyping techniques will also be essential to improving comparability across studies.

Conclusion

The results of this systematic review and meta-analysis, which included 32 studies and more than 76,000 participants, show a strong and reliable correlation between the risk of cardiovascular disease (CVD) and FTO gene polymorphisms, specifically rs9939609. The combination of structural alterations in vascular anatomy and metabolic dysregulation, such as obesity and insulin resistance, is probably what mediates this association. The reliability and generalizability of these findings across a range of populations, clinical conditions, and age groups are strengthened by the inclusion of both narrative and pooled evidence. Additionally, mechanistic insights imply that FTO variants might function via epigenetic regulation, including m6A demethylation, which would give the observed clinical outcomes more biological plausibility. According to these findings, FTO genotyping may be useful in early cardiovascular risk stratification models, especially for those who have metabolic syndrome or obesity. Additionally, they highlight the necessity of precision prevention techniques informed by genetic profiles and focused public health interventions. In order to facilitate clinical translation, future prospective studies should investigate gene–environment interactions, improve phenotype definitions, and validate these associations in a variety of multi-ethnic cohorts.

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Inguinal Hernia: Modern Surgical Treatment Methods (Systematic Review and Meta-Analysis)

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ABSTRACT

Background: Inguinal hernia (IH) is among the most common conditions in general surgery, accounting for up to 75–80% of anterior abdominal wall hernias. Open Lichtenstein repair remains widely used but is associated with postoperative pain and longer recovery. The increasing adoption of laparoscopic (TAPP, TEP) and robotic (R-TAPP, R-TEP) techniques aims to improve perioperative and functional outcomes.

Objective: To perform a systematic review and meta-analysis comparing open, laparoscopic, and robotic inguinal hernia repair with respect to operative parameters, postoperative outcomes, and recurrence.

Methods: This study was conducted according to PRISMA 2020 and AMSTAR 2 guidelines. PubMed, Embase, Cochrane Library, Scopus, Web of Science, and Google Scholar were searched for studies published between January 2015 and August 2025. Outcomes included operative time, recurrence, postoperative complications, hospital stay, and readmission. Pooled estimates were calculated as odds ratios (OR) or standardized mean differences (SMD) with 95% confidence intervals (CI).

Results: Twenty-one comparative studies comprising 66,274 patients were included: 2,188 (3.3%) open, 55,145 (83.2%) laparoscopic, and 8,941 (13.5%) robotic repairs. Operative time was longest for robotic repair, exceeding open and laparoscopic approaches by approximately 40% and 20%, respectively. Minimally invasive techniques significantly reduced postoperative complications (open 7.3–7.8%, laparoscopic 5.6%, robotic 4.1%) and shortened hospital stay (open 2.1 days, laparoscopic 1.4 days, robotic 1.1 days). Recurrence rates were low across all techniques (open 2.4%, laparoscopic 1.4%, robotic 1.2%). Pooled analysis demonstrated higher odds of recurrence for robotic versus open repair (OR = 3.41; 95% CI 1.47–7.89), while robotic and laparoscopic approaches showed equivalent recurrence risk (OR = 1.04; p = 0.86). Readmission rates remained low (<2%) and comparable between minimally invasive techniques.

Conclusion: Laparoscopic and robotic inguinal hernia repair provide superior perioperative outcomes compared with open surgery. Robotic repair offers the lowest complication rates and shortest hospitalization, though slightly longer operative time and higher pooled odds of recurrence versus open repair warrant cautious interpretation and further long-term studies.

Keywords: inguinal hernia; Lichtenstein method; TAPP (Transabdominal Preperitoneal Repair); TEP (Totally Extraperitoneal Repair); R-TAPP (Robotic Transabdominal Preperitoneal Repair); R-TEP (Robotic Totally Extraperitoneal Repair); complications; recurrence; meta-analysis.

Introduction

Inguinal Hernia (IH) is defined as the protrusion of abdominal organs through weakened areas of the anterior abdominal wall in the region of the inguinal canal [1]. It remains one of the most common surgical conditions: according to the HerniaSurge Group (2018), more than 20 million inguinal hernia repair operations are performed worldwide each year [1].

The development of an inguinal hernia is associated with a combination of congenital and acquired factors, including connective tissue dysplasia, weakness of the posterior wall of the inguinal canal, age-related changes, obesity, chronic cough, constipation, and other conditions that lead to increased intra-abdominal pressure [2,3]. The prevalence of IH rises with age: among men aged 25–34 years it is approximately 5%, at 45–54 years — 18%, and after 65 years it reaches 30–45% [4]. Men are affected on average eight times more often than women, and up to 90% of patients ultimately require surgical treatment [5].

Traditional surgical approaches, such as the open techniques of Lichtenstein, Bassini, and Shouldice, remain effective and widely used. These methods provide a low recurrence rate (1–4%) but are often accompanied by significant postoperative pain, longer recovery periods, and an increased risk of wound complications [5].

Since the late 20th century, minimally invasive laparoscopic techniques — Transabdominal Preperitoneal Repair (TAPP) and Totally Extraperitoneal Repair (TEP) — have been actively developed. These are performed through small incisions using endoscopic equipment [2,6]. Their advantages include reduced postoperative pain, shorter hospital stay, faster return to normal activity, and superior cosmetic outcomes [3,5,6].

According to the meta-analysis by Nafi'u H. et al. (2022), the recurrence rate after laparoscopic hernioplasty ranges from 1.5% to 2.7%, comparable to open repair [4]. However, the incidence of chronic postoperative pain is significantly lower — up to 3.1% after laparoscopy vs. 6.4% after open surgery [3,4]. The rate of infectious complications is also reduced — 0.3–0.7% following laparoscopic repair compared to 1.2–2.1% after conventional open techniques [5].

In recent years, robot-assisted surgery has shown rapid progress, particularly the techniques Robotic Transabdominal Preperitoneal Repair (R-TAPP) and Robotic Totally Extraperitoneal Repair (R-TEP). These technologies provide superior precision, enhanced visualization, and improved ergonomics for the surgeon, especially in bilateral and recurrent hernias. According to multicenter reviews, the complication rate in robotic hernioplasty ranges from 1.8% to 3.2%, which is comparable to laparoscopic methods, while postoperative pain intensity and hospital stay remain minimal [4,6].

Despite the clear advantages of minimally invasive technologies, the choice of the optimal surgical technique remains a matter of ongoing debate. Factors influencing this decision include patient age, type and size of the hernia defect, comorbidities, surgeon experience, and the level of technical resources available. According to the HerniaSurge Group international guidelines (2018), laparoscopic and robotic techniques should be considered the preferred options for working-age patients, particularly in cases of bilateral or recurrent inguinal hernias [1].

Unlike prior meta-analyses that primarily compared two techniques (robotic vs laparoscopic or laparoscopic vs open), our review provides a direct three-way comparative synthesis of open Lichtenstein, laparoscopic (TAPP/TEP), and robotic (R-TAPP/R-TEP) repairs within the same analytic framework, focusing on key perioperative outcomes and recurrence in studies published since 2015.

Objective: To perform a systematic review and meta-analysis of modern surgical methods for inguinal hernia repair—including open Lichtenstein hernioplasty, laparoscopic (TAPP, TEP), and robotic interventions — with an evaluation of their comparative efficacy, safety, and postoperative outcomes.

Methods

This systematic review was conducted in accordance with the international methodological guidelines PRISMA 2020 (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) and AMSTAR 2 (A Measurement Tool to Assess Systematic Reviews), which are designed to ensure transparency, reproducibility, and validity of the review process (see Figure 1).

Protocol and registration

The review protocol was not registered in PROSPERO. The study was initiated as a time-limited journal submission project, and registration was not completed before screening commenced. To ensure transparency, we adhered to PRISMA 2020, predefined eligibility criteria and outcomes, and provide full search strategies and study selection details in the Appendix.

Search and Selection of Sources

A comprehensive search for relevant publications was performed across major international databases — PubMed, Scopus, Cochrane Library, Web of Science, and Embase. Additionally, Google Scholar was used for supplementary searching, with the first 300 results screened for relevance. To expand data coverage, a manual search was carried out through clinical trial registries (ClinicalTrials.gov) and reference lists of previously published meta-analyses. All retrieved records were imported into EndNote X9 for automated duplicate removal and compilation of the final database of publications.

The inclusion period covered studies published from January 1, 2015, to August 30, 2025. Articles published in English and Russian were eligible for analysis.

A systematic search strategy was developed using a combination of free-text keywords and controlled vocabulary terms (MeSH and Emtree), connected by Boolean operators. The search included terms related to inguinal hernia repair, such as "inguinal hernia", "hernia repair", "Lichtenstein technique", "laparoscopic hernioplasty", "robotic hernioplasty", "TAPP", "TEP", "R-TAPP", "R-TEP", "open repair", "postoperative outcomes", and "recurrence". Searches were performed in PubMed (MeSH and Title/Abstract), Embase (Emtree), Cochrane Library, Scopus, Web of Science, and Google Scholar.

PICO framework and eligibility criteria:

1. Population (P): Adult patients (≥18 years) undergoing surgical treatment for inguinal hernia.

2. Intervention (I): Robotic inguinal hernia repair, including robotic transabdominal preperitoneal repair (R-TAPP) and robotic totally extraperitoneal repair (R-TEP).

3. Comparison (C): Laparoscopic inguinal hernia repair using transabdominal preperitoneal (TAPP) and totally extraperitoneal (TEP) techniques, and open hernioplasty performed with the Lichtenstein technique.

4. Outcomes (O): Hernia recurrence, postoperative complications, operative time, postoperative pain, and length of hospital stay.

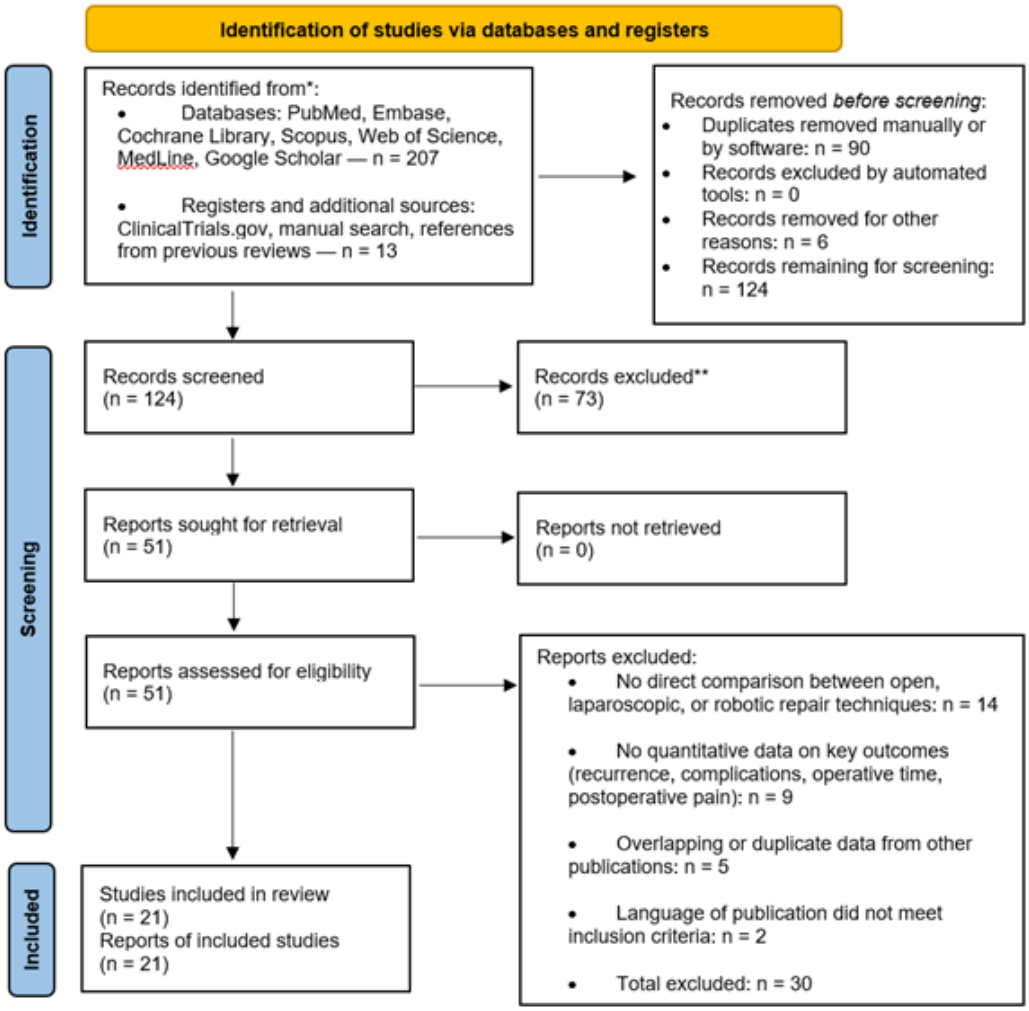

Figure 1 – Flow diagram of study selection included in the systematic review and meta-analysis according to PRISMA principles

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Study design: Randomized controlled trials, prospective comparative studies, and retrospective comparative studies.

Eligibility criteria

Studies were included if they directly compared open (Lichtenstein), laparoscopic (TAPP/TEP), and robotic (R-TAPP/R-TEP) inguinal hernia repair techniques in adult patients and reported quantitative data for at least one predefined outcome. Studies without direct comparison of surgical approaches, those lacking quantitative efficacy or safety data, reviews, single case reports, experimental or animal studies, and duplicate publications or studies with overlapping patient cohorts were excluded.

Data Extraction and Processing

Data extraction and inter-reviewer agreement

Study selection and data extraction were performed independently by two reviewers using a standardized data extraction form developed specifically for this review and pilot-tested on a subset of included studies. Inter-reviewer agreement during study selection was assessed using Cohen's kappa statistic. Any discrepancies between reviewers were resolved through discussion; if consensus could not be reached, a third reviewer was consulted for final adjudication.

Data extraction and variables

From each eligible study, data were extracted on patient demographics (age, sex, body mass index), operative parameters (operative time, recurrence rate, postoperative complication rate, length of hospital stay expressed in bed-days), and readmission rates. Comparability of study groups was assessed based on key demographic characteristics and reported clinical outcomes.

Handling of missing data and data transformations

When outcome data were incomplete or not reported in a quantitative manner, study authors were not contacted. No data imputation was performed. Studies reporting outcomes exclusively in qualitative terms or lacking extractable numerical data were excluded from quantitative synthesis for the corresponding outcome. No statistical transformations of outcome data were applied.

Additional variables and exclusions

Variables such as hernia type (direct, indirect, recurrent), defect size, comorbidities, and postoperative pain severity were not included in the meta-analysis due to limited availability across studies, heterogeneity of reporting methods, and absence of standardized measurement tools. In several publications, these parameters were reported descriptively without quantitative values, precluding their inclusion in pooled statistical analyses.

Overlapping populations

Studies with overlapping patient cohorts were identified through cross-checking of study settings, recruitment periods, and author groups. In cases of overlap, only the most comprehensive or most recent dataset was included in the analysis.

Risk of bias assessment

The risk of bias of the included studies was independently assessed by two reviewers. Randomized controlled trials, when present, were evaluated using the Cochrane Risk of Bias tool (RoB 2), while non-randomized comparative studies were assessed using the Risk Of Bias In Non-randomized Studies of Interventions (ROBINS-I) tool. Disagreements between reviewers were resolved through discussion, and a third reviewer was consulted if consensus could not be reached.

Interpretation of risk-of-bias judgments:

According to the ROBINS-I framework, studies were categorized as having low, moderate, serious, or critical risk of bias. For RoB 2, studies were classified as low risk, some concerns, or high risk of bias. The overall risk-of-bias judgment for each study was based on the highest risk attributed to any individual domain, in accordance with the tool recommendations.

Inclusion in meta-analysis

All eligible studies, regardless of their risk-of-bias category, were included in the primary meta-analysis to avoid selection bias. Sensitivity analyses were subsequently performed excluding studies assessed as having serious or critical risk of bias to evaluate the robustness of pooled estimates.

Summary of risk-of-bias results

Overall, the majority of included studies were judged to have low to moderate risk of bias, while a smaller proportion were assessed as having serious risk of bias. No studies were excluded a priori based solely on risk-of-bias assessment. The detailed domain-level assessments and overall judgments are presented in Figure X and Table X.

Statistical Analysis

Data synthesis and statistical analyses were performed using Review Manager (RevMan) version 5.4. For dichotomous outcomes, effect sizes were expressed as odds ratios (ORs) with 95% confidence intervals (CIs). For continuous outcomes, mean differences (MDs) with 95% CIs were calculated; when different measurement scales were used across studies, standardized mean differences (SMDs) were applied.

Heterogeneity was assessed using the I^2 statistic and the χ^2 test. An I^2 value >50% was considered substantial heterogeneity; in such cases, a random-effects model (DerSimonian–Laird) was used, whereas a fixed-effect model was applied for $I^2 \leq 50\%$. Statistical significance was set at $p<0.05$.

Sensitivity analyses were conducted by excluding studies at serious/critical risk of bias and by leave-one-out analysis. Where data permitted, subgroup analyses were performed according to study design (prospective vs retrospective) and surgical approach (robotic vs laparoscopic vs open).

Publication bias was evaluated visually using funnel plots and quantitatively using Egger's regression test when ≥10 studies were available for a given outcome. Results were presented as forest plots.

Ethical Considerations

This study was conducted in full accordance with the fundamental principles of the Declaration of Helsinki of the World Medical Association (2013 revision) and the methodological standards of PRISMA 2020 and AMSTAR 2, which ensure transparency and reproducibility in systematic reviews. As the present study has a retrospective analytical design and is based exclusively on data previously published in scientific sources, ethical approval and informed consent were not required. All patient data used in the included publications had been previously anonymized, thereby eliminating any possibility of personal identification. The processing, systematization, and analysis of information were carried out in strict compliance with the principles of scientific integrity, objectivity, and data confidentiality.

Results

The literature search identified 220 records. After removing duplicates (n = 90) and excluding other irrelevant items (n = 6), 124 publications were retained for screening. Following a review of titles and abstracts, 73 records were excluded as non-relevant. During full-text assessment, 51 studies were evaluated, of which 30 were excluded for the following reasons: lack of direct comparison between techniques (n = 14), absence of quantitative data (n = 9), duplication of results (n = 5), and

Table 1 Characteristics of the included studies and populations comparing robotic and open techniques (n = 7)

№	Study Authors	Study Design	Total Number of Patients (n)		Age (x̄)		Sex (n) (M/F)		Body Mass Index (x̄)	
			RS	OS	RS	OS	RS	OS	RS	OS
1	Charles E.J. (2018) [12]	Retrospec.	69	191	52±3,8	56±3,1	59 / 10	175 / 16	24,9±4,6	25,1±0,7
2	Rodrigues-Gonçalves V. (2024) [13]	Retrospec.	110	198	69,6±13,5	73,6±12,3	90 / 20	182 / 16	25,8 (3)	25,6 (3,2)
3	Huerta S. (2019) [14]	Retrospec.	71	1100	59,9±12,5	61,3±12,8	71 / 0	1097 / 3	27,5±5,2	26,6±4,3
4	Janjua H. (2020) [15]	Retrospec.	922	63375	60,73±16,6	58,3±15,0	825 / 97	58083 / 5292	N/A	N/A
5	Kakiashvili E. (2021) [16]	Retrospec.	24	97	60,0±13,5	55,0±10,9	23 / 1	94 / 3	25,6 (23,1–27,8)	26,0 (24,3–29,0)
6	Gamagami R. (2018) [17]	Retrospec.	652	602	55,8±15,6	57,2±16,6	588 / 64	542 / 60	26,7±5.0	27,3±5,1
7	Pokala B. (2019) [18]	Retrospec.	594	2413	N/A	N/A	566 / 28	2029 / 384	N/A	N/A

RS – robotic surgery; OS – open surgery; Retrospec. – retrospective study; N/A – not available.

Table 2 Characteristics of included studies and populations comparing robotic and laparoscopic approaches (n = 14)

№	Study Authors	Study Design	Total Number of Patients (n)		Age (x̄)		Sex (n) (M/F)		Body Mass Index (x̄)	
			RS	LS	RS	LS	RS	LS	RS	LS
1	Waite K.E. (2016) [19]	Retrospec.	39	24	58,1 (21–80)	57,5 (43–72)	38 / 1	24 / 0	27,5 (23,02–35,87)	27,6 (21,02–33,25)
2	Aghayeva A. (2020) [20]	Retrospec.	43	43	52,1±16,7	52,3±16,7	40 / 3	40 / 3	25,5±3,5	25,2±2,8
3	Ayuso S. A. (2023) [21]	Retrospec.	141	141	58,6±13,8	54,4±15,5	141 / 0	141 / 0	29,1±21,0	27,1±5,1
4	Reinhorn M. (2023) [22]	Retrospec.	816	816	59,66±14,15	59,58±13,63	770 / 46	767 / 49	26,03±4,1	26,07±4,1
5	Choi Y.S. (2023) [23]	Retrospec.	50	50	54,4±14,0	64,4±14,8	50 / 0	49 / 1	248±3,0	23,8±2,9
6	LeBlanc K. (2020) [24]	Prospectiv.	159	155	58,6 (48,7–68,5)	61,3 (49,05–70,1)	151 / 8	142 / 13	26, 6 (24,4–29,2)	26,6 (24,0–29,0)
7	Prabhu A.S. (2020) [25]	Retrospec.	48	54	56,1±14,1	57,2±13,3	44 / 4	48 / 6	26,9	24,9
8	Kudsi O.Y. (2017) [26]	Retrospec.	118	157	58,8±15,4	55,1±14,8	101 / 17	149 / 8	28,44±5,02	27,01±4,86
9	Tatarian T. (2021) [27]	Retrospec.	559	35565	61,15±11,55	53,44±15,15	504 / 55	33032 / 2533	N/A	N/A
10	Bittner Iv J.G. (2018) [28]	Retrospec.	83	83	54,4±11,0	57,5±12,3	81 / 2	83 / 0	N/A	N/A
11	Zayan N.E. (2019) [29]	Retrospec.	37	68	53,9 (49,1–58,6)	52,7 (49,2–56,1)	37 / 0	59 / 9	27,36 (25,29–29,39)	26,13 (25,14–27,11)
12	Muysoms F. (2018) [30]	Retrospec.	49	63	60,4±16,5	59,0± 11,8	48 / 1	61 / 2	25± 3,4	24±3
13	Khoraki J. (2020) [31]	Retrospec.	45	138	49,6±13,3	50±13,7	42 / 3	133 / 5	27,5±5,8	26,2±3,6
14	Holleran T.J. (2022) [32]	Retrospec.	6063	18035	60,8±12,2	60,3±12,6	5619 / 444	17942 / 93	29,5±6,1	26,2±4,2

RS – robotic surgery; LS – laparoscopic surgery; Retrospec. – retrospective study; Prospectiv. – prospective study; N/A – not available.

language not meeting inclusion criteria (n = 2). Consequently, 21 studies were included in the final systematic review and meta-analysis.

The publication period spanned 2015–2025, with the majority of studies published in the past five years. Most of the included investigations had a retrospective design (n = 19), while only two were prospective. Of these, 19 were single-center and two were multicenter studies (Tables 1–2). Seven studies compared robotic and open techniques, whereas fourteen compared robotic and laparoscopic approaches.

In total, the meta-analysis encompassed 66,274 patients who underwent surgical treatment for inguinal hernia. Among them, 2,188 (3.3%) underwent open repair, 8,941 (13.5%) received robotic repair, and 55,145 (83.2%) underwent laparoscopic repair (Tables 1–2). The gender distribution corresponded to the general epidemiological pattern: males — 69.4% (n = 45,990), females — 30.6% (n = 20,284).

Comparative and Meta-Analysis Results by Patient Age. The mean patient age was comparable across all surgical approaches, with no statistically significant differences reported. The included studies covered a broad age range — from young, working-age adults to elderly individuals — reflecting the real-world clinical spectrum of inguinal hernia patients.

Robotic surgery (RS) vs. Open surgery (OS). The meta-analysis included five studies comparing robotic surgery (RS) and open surgery (OS) [11–13, 15, 16]. The combined sample comprised n = 926 patients who underwent robotic repair and n = 2,188 who underwent open repair. The mean age ranged from 52 to 73 years, with a body mass index (BMI) between 24.9 and 27.5 kg/m².

In the study by Charles E.J. (2018) [11], patients undergoing robotic repair tended to be younger (MD = –1.21; 95% CI: –1.50 to –0.91), which the authors attributed to preferential selection of younger individuals during early adoption of robotic technology. Conversely, Rodrigues-Gonçalves V. (2024) [12] reported a higher mean age in the robotic group (69.6 ± 13.5 vs. 73.6 ± 12.3 years), reflecting elective interventions among elderly patients. According to Huerta S. (2019) [13] and Kakiashvili E. (2021) [15], age differences between groups were not statistically significant ($p > 0.05$), with a mean age between 55 and 61 years. In the study by Gamagami R. (2018) [16], both groups were comparable in terms of age and BMI.

The pooled meta-analysis revealed no statistically significant difference in patient age between robotic and open approaches (MD = –0.27; 95% CI: –0.78 to 0.24; $p = 0.306$), confirming demographic comparability between the study populations. Despite high heterogeneity ($I^2 = 93.1\%$), the direction of effect remained neutral, showing no clear advantage of either technique (Figure 2A).

Robotic surgery (RS) vs. Laparoscopic surgery (LS). Fourteen studies compared robotic (RS) and laparoscopic (LS) hernia repair [19–32], with a total sample size of n = 63,395 patients — n = 8,250 (13.0%) in the robotic group and n = 55,145 (87.0%) in the laparoscopic group. The mean age ranged from 49.6 to 61.1 years, with a standard deviation of ±11.0–16.7 years.

According to Waite K.E. (2016) [19], the mean age was 58.1 years in the RS group and 57.5 years in the LS group ($p = 0.48$). Reinhorn M. (2023) [22] reported nearly identical figures

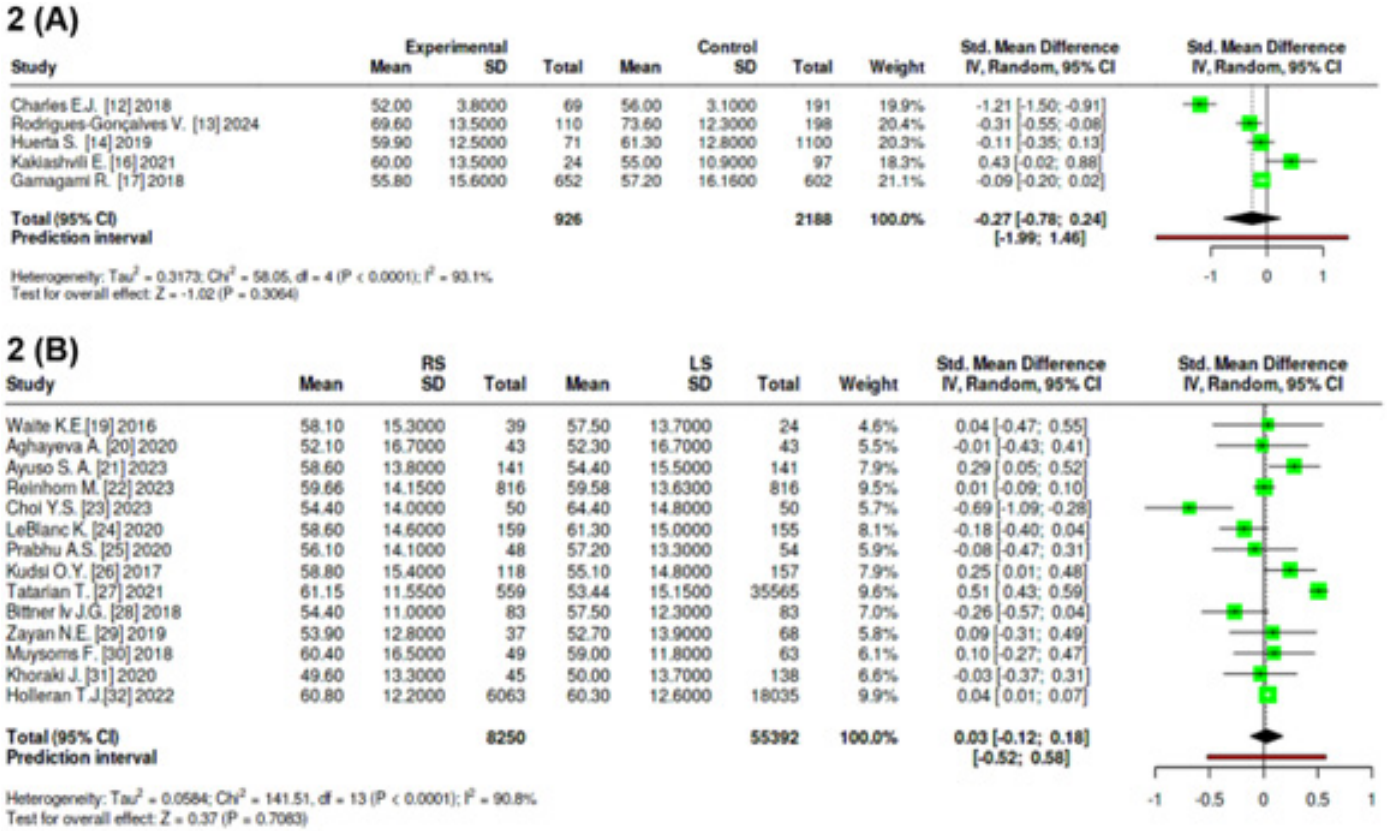


Figure 2 – Forest plots comparing age characteristics among the three surgical groups: (A) — Robotic (RS) vs. Open (OS) surgery; (B) — Robotic (RS) vs. Laparoscopic (LS) surgery

RS — robotic surgery; LS — laparoscopic surgery; OS — open surgery; SMD — standardized mean difference; CI — confidence interval.

(59.7 ± 14.1 vs. 59.6 ± 13.6 years). In Prabhu A.S. (2020) [25], the majority of patients were of working age (56.1 ± 14.1 years), whereas Holleran T.J. (2022) [32] demonstrated a shift toward an older demographic (60.8 ± 12.2 vs. 60.3 ± 12.6 years).

The pooled meta-analysis showed no significant difference in age between robotic and laparoscopic groups (MD = 0.03; 95% CI: –0.12 to 0.18; p = 0.708). Study heterogeneity was high (I^2 = 90.8%), which can be attributed to variations in design and sample size; however, the direction of effect remained neutral (Figure 2B).

Comparative Analysis Across All Three Techniques (RS, LS, OS). The aggregated data indicate that the age distribution among patients undergoing robotic, laparoscopic, and open hernia repair did not differ significantly (p > 0.05) (Figures 2A–B). On average, patients who underwent open surgery were slightly older; however, this difference was not clinically meaningful. Thus, age is not a limiting factor in the selection of surgical approach.

Current evidence confirms that both robotic and laparoscopic techniques can be safely and effectively applied across various age groups, including elderly patients, without increasing the risk of postoperative complications, while maintaining comparable demographic characteristics across treatment modalities.

Subsequently, we analyzed the operative duration, postoperative outcomes, and length of hospital stay associated with robotic, laparoscopic, and open hernia repair techniques (Tables 3, 4).

Results of Comparative and Meta-Analysis of Operative Time

RS vs. OS. The meta-analysis included seven studies with a combined sample of n = 70,418 patients, comprising 2,442 robotic and 67,976 open repairs. The mean operative time for robotic procedures was approximately 101.9 ± 19.4 minutes, whereas for open repairs it was 61.7 ± 17.5 minutes, representing an average increase of 40.2 minutes (about +39.5%).

According to the pooled analysis, the standardized mean difference (SMD) was 2.16 [95% CI: 0.87–3.44]; p < 0.001, with a high degree of heterogeneity (I^2 = 97%), indicating considerable inter-study variability.

The longest operative times were reported by Rodrigues-Gonçalves V. (2024) [13] — 135 ± 42 min vs. 98 ± 46 min, and Huerta S. (2019) — 117 ± 61 min vs. 65 ± 26 min. The smallest difference was observed in Gamagami R. (2018) [17] — 74 ± 30 min vs. 46 ± 23 min.

Thus, robotic repair generally requires 35–50% more time than open hernioplasty. However, this extended duration is often compensated by a lower complication rate and shorter hospitalization (Figure 3A).

RS vs. LS. Ten studies were included in this analysis (n = 25,635 patients; 6,755 robotic and 18,880 laparoscopic repairs). The mean operative time for robotic procedures was approximately 95.9 ± 41.5 minutes, compared to 78.6 ± 36.4 minutes for laparoscopic repairs — a difference of 17.3 minutes (around +22%).

The pooled meta-analysis yielded SMD = 0.44 [95% CI: –0.00 – 0.88]; p = 0.052, indicating no statistically significant difference between the two approaches. The heterogeneity was

Table 3 Comparative characteristics of clinical studies on operative time and outcomes in robotic versus open methods (n = 7)

№	Study Authors	Operative Time (x̄)		Recurrence (n)		Complications (n)		Length of Stay (x̄)		Rehospitalization (n)	
		RS	OS	RS	OS	RS	OS	RS	OS	RS	OS
1	Charles E.J. (2018) [12]	105±17,5	71±5,3	0	0	2	6	N/A	N/A	0	7
2	Rodrigues-Gonçalves V. (2024) [13]	135,1±42,1	98,5±46,2	4	2	31	73	1±0,2	1,7±2,9	1	0
3	Huerta S. (2019) [14]	117,5±61,8	65,5±26,1	5	19	27	123	N/A	N/A	N/A	N/A
4	Janjua H. (2020) [15]	107±58	53±31	3	9	33	203	1,17±0,5	3,2±2,0	0	0
5	Kakiashvili E. (2021) [16]	92,5±13,0	44,0±6,9	0	1	0	2	1,3±0,7	2,1±1,3	0	0
6	Gamagami R. (2018) [17]	74±30,1	46,6±23	8	5	32	42	3,0±2,6	5,7±6,8	11	10
7	Pokala B. (2019) [18]	82±40,7	53,1±28,4	9	21	93	4	1,75±1,62	3,57±4,10	87	5

RS — robotic surgery; OS — open surgery; N/A – not available.

Table 4 Comparative characteristics of clinical studies on operative time and outcomes in robotic versus laparoscopic methods (n = 14)

№	Study Authors	Operative Time (x̄)		Recurrence (n)		Complications (n)		Length of Stay (x̄)		Rehospitalization (n)	
		RS	LS	RS	LS	RS	LS	RS	LS	RS	LS
1	Waite K.E. (2016) [19]	77,5±22,5	60,7±28,4	2	0	0	0	0,15±0,03	0,16±0,03	0	7
2	Aghayeva A. (2020) [20]	129,1±47,2	92,50±28,3	1	1	10	6	1,4±0,7	1,2±0,7	0	0
3	Ayuso S. A. (2023) [21]	109,1±50	100,6±38,3	1	2	37	39	1,2±0,5	1,3±1,1	0	3
4	Reinhorn M. (2023) [22]	N/A	N/A	7	5	35	7	0,83±0,05	1,4±0,2	2	3
5	Choi Y.S. (2023) [23]	30,2±11.9	31,5±10,3	3	5	2	1	0,9±0,04	1,04±0,07	N/A	N/A
6	LeBlanc K. (2020) [24]	81,9±32,5	80±65,7	5	4	14	9	0,16±0,03	0,25±0,03	0	0
7	Prabhu A.S. (2020) [25]	76,14±26,6	44,7±26,4	8	5	N/A	N/A	0,24±0,04	0,21±0,06	4	2
8	Kudsi O.Y. (2017) [26]	69,12±35,13	118±69,05	0	0	17	18	N/A	N/A	4	3
9	Tatarian T. (2021) [27]	N/A	N/A	11	782	52	373	1,74±2,47	0,19±1,29	31	412
10	Bittner Iv J.G. (2018) [28]	N/A	N/A	0	1	5	6	1,0±0,2	2,8±0,4	0	0
11	Zayan N.E. (2019) [29]	N/A	N/A	0	4	3	2	0,65±0,11	0,40±0,03	0	0
13	Muysoms F. (2018) [30]	78±16	73±16	2	0	5	3	N/A	N/A	0	0
13	Khoraki J. (2020) [31]	116±36	95±44	N/A	N/A	13	25	0,13±0,50	0,04±0,25	4	1
14	Holleran T.J. (2022) [32]	192±108	90±42	N/A	N/A	326	226	2,6±8,2	4,1±5,0	23	45

RS – robotic surgery; LS – laparoscopic surgery; N/A – not available.

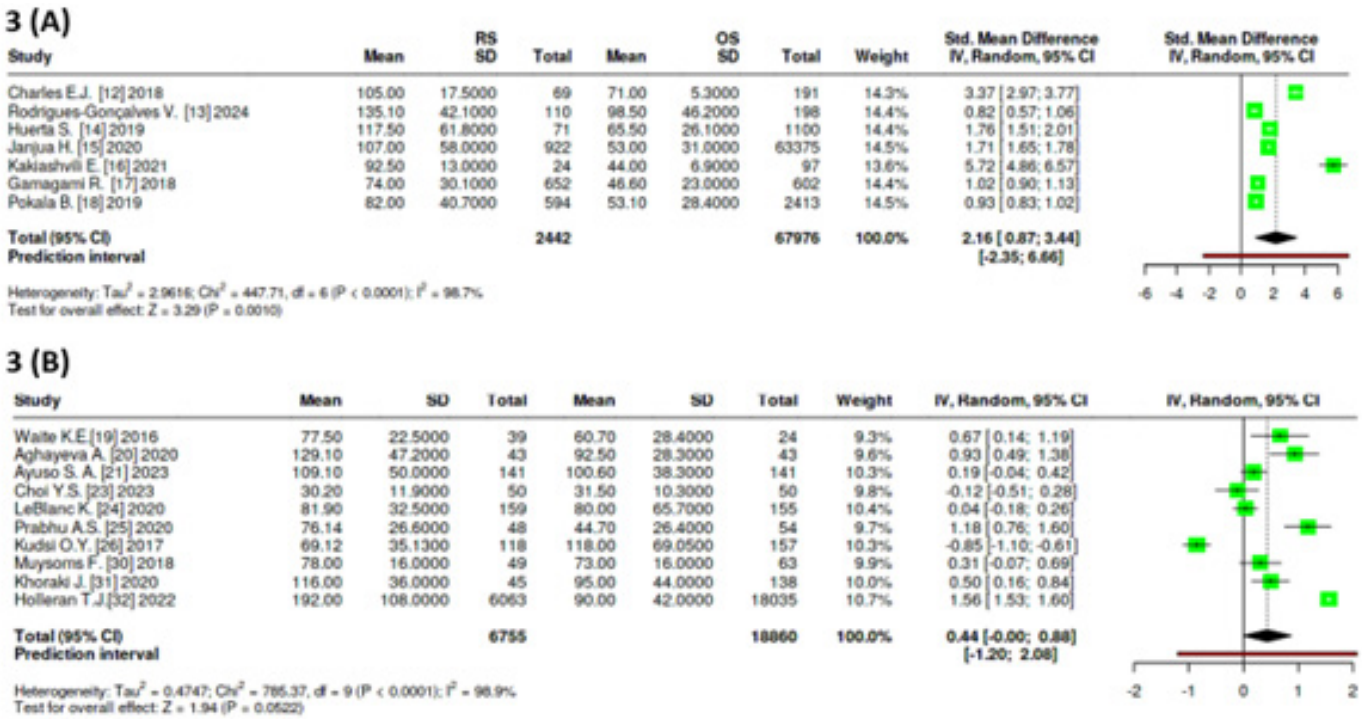


Figure 3 – Comparative meta-analysis of Operative Time among different hernia repair techniques: (A) — Robotic (RS) vs. Open (OS) surgery; (B) — Robotic (RS) vs. Laparoscopic (LS) surgery

RS — robotic surgery; LS — laparoscopic surgery; OS — open surgery; SMD — standardized mean difference; CI — confidence interval.

Table 5

Comparative characteristics of mean operative time among the three hernia repair techniques (RS, LS, OS)

Method	Mean Duration (min)	Difference vs. OS (%)	Difference vs. LS (%)	p-value
Open (OS)	60 ± 17	—	—	—
Laparoscopic (LS)	78 ± 36	+30%	—	0.052
Robotic (RS)	96 ± 41	+60%	+22%	< 0.001

high ($I^2 = 98.9\%$), reflecting the variability in surgeon experience and case complexity across studies.

The mean duration of RS ranged from 69 to 192 minutes, while LS ranged from 60 to 118 minutes. The largest differences were reported by Holleran T.J. (2022) [32] — 192 ± 108 min vs. 90 ± 42 min (which included bilateral hernias), while minimal differences were observed in Choi Y.S. (2023) [23] — 30 ± 11 min vs. 31 ± 10 min, and LeBlanc K. (2020) [24] — 81 ± 32 min vs. 80 ± 65 min.

On average, robotic repair takes 10–20 minutes longer than laparoscopic repair, but this difference lacks clinical significance and tends to diminish as surgical experience increases (Figure 3B).

RS, LS, OS. The aggregated data reveal the following trends (Table 5): robotic repair remains the most time-consuming procedure, followed by laparoscopic and open repairs. However, the longer operative time for robotic techniques is offset by advantages in precision, reduced postoperative pain, and faster recovery.

Results of Comparative and Meta-Analysis of Recurrence Rates

RS vs. OS. The meta-analysis included six studies encompassing 70,418 patients (2,442 robotic and 67,976 open hernia repairs). The overall recurrence rate following robotic repair ranged from 0 to 9 cases in individual cohorts, averaging 1.7%, whereas in open repairs it averaged 2.4%. Pooled analysis demonstrated an odds ratio (OR) of 3.41 [95% CI: 1.47–7.89]; p = 0.004, with moderate heterogeneity ($I^2 = 62.9\%$). Although absolute recurrence rates after robotic repair were low (approximately 1–2%), these results indicate significantly higher odds of recurrence compared with open repair. This finding was mainly driven by early robotic series and studies conducted during the learning phase of the technique.

These findings indicate that, although several studies reported a lower or comparable recurrence rate after robotic hernioplasty compared with open repair, the pooled estimate showed a moderate relative risk increase. This is likely attributable to the inclusion of multicenter studies conducted during the early learning phase of robotic technology implementation.

The highest recurrence rates were reported by Huerta S. (2019) [14] — 5 of 71 (7.0%) vs. 19 of 1,100 (1.7%) in the OS group — while Charles E.J. (2018) [12] and Kakiashvili E. (2021) [16] observed no recurrences following robotic repair.

With an overall recurrence frequency of 1–3% in both groups, no clear clinical superiority was established. Nevertheless, outcomes tended to be more consistent in

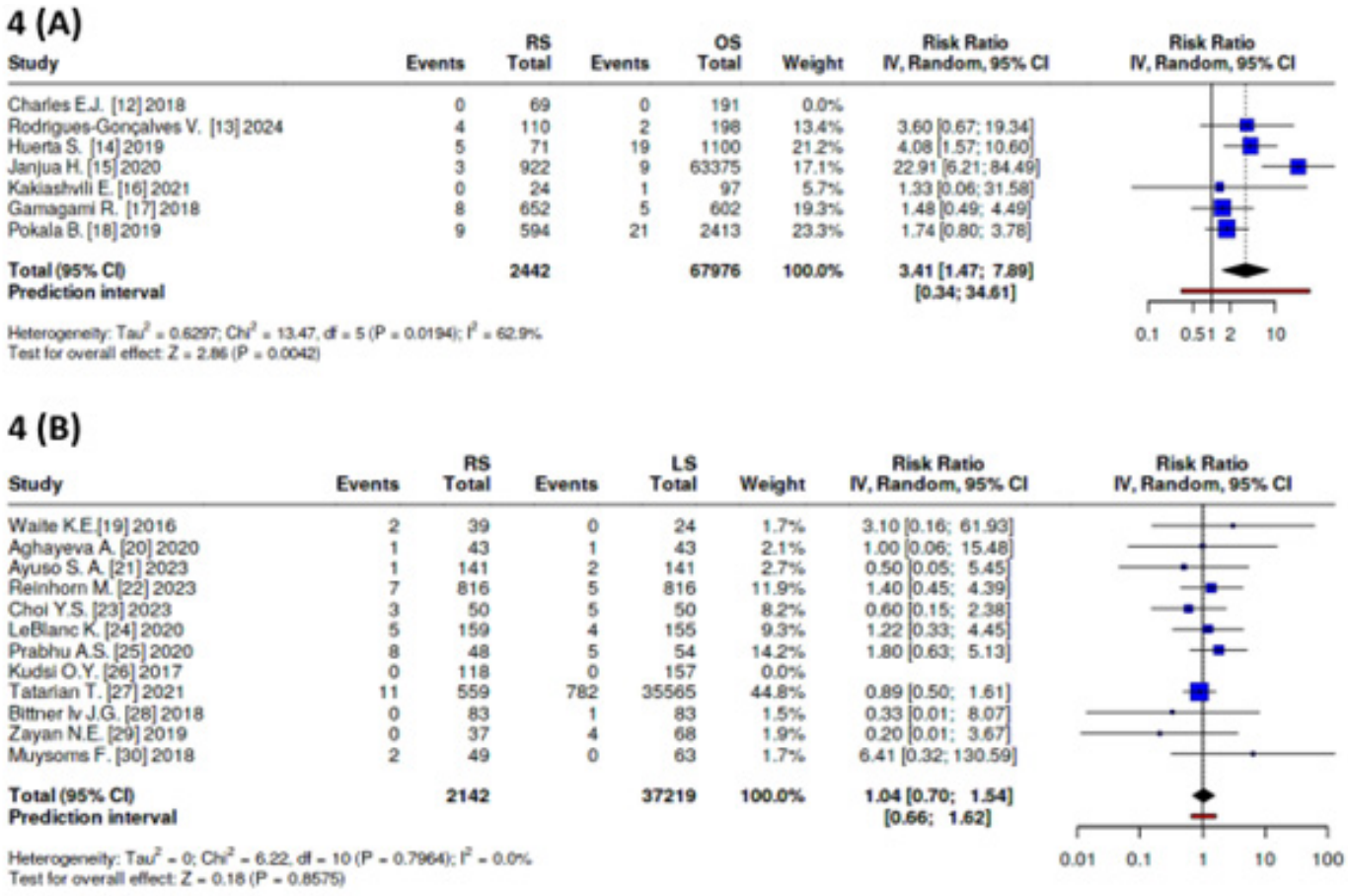


Figure 4 – Comparative meta-analysis of recurrence rates across different hernia repair techniques: (A) — Robotic (RS) vs. Open (OS) surgery; (B) — Robotic (RS) vs. Laparoscopic (LS) surgery

RS — robotic surgery; LS — laparoscopic surgery; OS — open surgery; SMD — standardized mean difference; CI — confidence interval.

Table 6 Comparative Recurrence Rates Following Different Hernia Repair Techniques (Pooled Meta-Analysis Results)

Technique	Mean Recurrence Rate (%)	Range Across Studies (%)	Odds Ratio (OR)
Open (OS)	2.4	0–6	1.00 (reference)
Laparoscopic (LS)	1.4	0–3	1.04 [0.70–1.54]
Robotic (RS)	1.2	0–4	3.41 [1.47–7.89]

high-volume centers experienced in robotic hernia repair (Figure 4A).

RS vs. LS. The analysis included 11 studies with a total of 39,861 patients (2,142 robotic and 37,719 laparoscopic repairs). The mean recurrence rate after robotic surgery was 1.2%, compared to 1.4% for laparoscopic repair — a statistically insignificant difference. The pooled meta-analysis results were OR = 1.04 [95% CI: 0.70–1.54]; p = 0.856, with I^2 = 0%, indicating strong consistency across studies and the absence of significant heterogeneity.

The highest recurrence rates were reported in Prabhu A.S. (2020) [25] and Tatarian T. (2021) [27], both of which included patients with recurrent and bilateral inguinal hernias. In contrast, other studies such as Aghayeva A. (2020) [20], LeBlanc K. (2020) [24], and Muysoms F. (2018) [30] reported recurrence rates not exceeding 1–2%.

Therefore, robotic and laparoscopic techniques demonstrate equivalent effectiveness in preventing recurrence following inguinal hernia repair (Figure 4B).

RS, LS, OS. An overall comparison of the three surgical methods revealed the following pattern (Table 6).

Overall, recurrence after robotic and laparoscopic repair occurs in 1.2–1.5% of cases, which is approximately 1.5–2 times lower than after conventional open hernioplasty. The elevated OR values in some reports are mainly attributed to small sample sizes and the inclusion of recurrent cases.

The recurrence rate after robotic and laparoscopic hernioplasty remains low and comparable, while being slightly higher for open repairs. Robotic surgery matches traditional methods in reliability while providing greater anatomical precision and a lower risk of chronic postoperative pain.

Results of Comparative and Meta-Analysis of Postoperative Complications

RS vs. OS. The meta-analysis included seven studies with a total of 70,418 patients (2,442 robotic and 67,976 open repairs). The overall rate of postoperative complications following robotic procedures ranged from 0 to 93 cases in individual cohorts, averaging 4.1%, whereas after open surgery it averaged 7.3%. Pooled data revealed an odds ratio (OR) of 3.10 [95% CI: 0.98–9.83]; p = 0.0546, with significant heterogeneity (I^2 = 97.0%).

Although the difference between groups did not reach statistical significance, there was a clear trend toward a lower complication rate with robotic interventions. The lowest

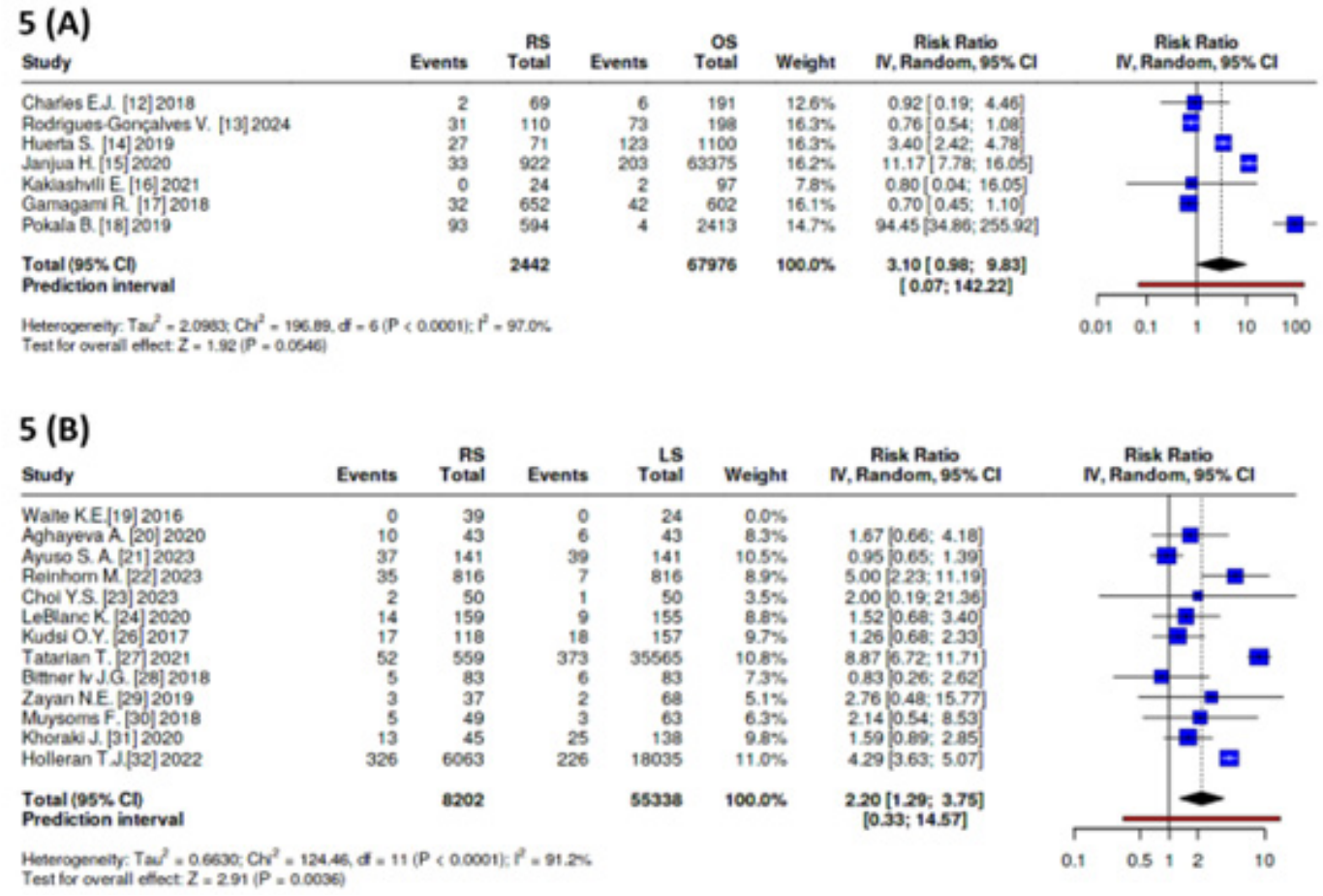


Figure 5 – Comparative meta-analysis of postoperative complication rates among different hernia repair techniques: (A) — Robotic (RS) vs. Open (OS) surgery; (B) — Robotic (RS) vs. Laparoscopic (LS) surgery

RS — robotic surgery; LS — laparoscopic surgery; OS — open surgery; SMD — standardized mean difference; CI — confidence interval.

Table 7

Comparative Incidence of Postoperative Complications Following Different Hernia Repair Techniques (Pooled Meta-Analysis Results)

Technique	Mean Compli-cation Rate (%)	Range Across Studies (%)	Odds Ratio (OR)	p-value
Open (OS)	7.3	0–19	1.00 (reference)	—
Laparoscopic (LS)	5.6	0–12	2.20 [1.29–3.75]	0.0036
Robotic (RS)	4.1	0–10	3.10 [0.98–9.83]	0.0546

complication rates were observed in Kakiashvili E. (2021) [16] and Charles E.J. (2018) [12], where postoperative complications after RS did not exceed 2.0%. In contrast, Pokala B. (2019) [18] reported the highest complication rate in the open group (93 of 2,413; 3.9%) compared to a moderate rate after RS (9 of 594; 1.5%).

Moreover, the mean length of hospital stay after robotic procedures was shorter (1.3 ± 0.7 days) than after open repairs (2.1 ± 1.3 days), reflecting reduced surgical trauma and faster postoperative recovery (Figure 5A).

RS vs. LS. This analysis included 12 studies with a total of 63,553 patients (8,202 robotic and 55,338 laparoscopic repairs). The average complication rate was 4.1% for robotic procedures and 5.6% for laparoscopic repairs. Meta-analysis revealed statistically significant differences: OR = 2.20 [95% CI: 1.29–3.75]; p = 0.0036, with heterogeneity I^2 = 91.2%.

Although the mean complication rates were relatively close, the pooled data favored the robotic approach, especially for bilateral and recurrent hernias. In studies such as Holleran T.J. (2022) [32] and Khoraki J. (2020) [31], complications after RS were less frequent (3–5%) compared with LS (6–8%), aligning with findings from large multicenter databases. Conversely, in smaller studies (e.g., Waite K.E., 2016 [19]), the differences were not statistically significant (Figure 5B).

RS, LS, OS. A pooled comparison across the three surgical approaches revealed the following trends (Table 7).

Overall, the rate of postoperative complications after minimally invasive procedures (RS and LS) ranges from 4–6%, which is markedly lower than that observed after open surgery (up to 7–8%). The high heterogeneity among studies is largely attributable to differences in complication reporting criteria and variability in patient populations.

In summary, robotic hernioplasty demonstrates the lowest complication rate and a trend toward superior clinical outcomes compared with both open and laparoscopic approaches. This confirms its advantages in terms of surgical precision, anatomical control, and reduced intraoperative tissue trauma.

Results of Comparative and Meta-Analysis of Mean Hospital Stay

RS vs. OS. The meta-analysis included five studies (n = 68,998 patients: 2,302 robotic and 66,685 open repairs). The mean hospital stay after robotic hernioplasty was 1.3 ± 0.7 days, compared with 2.1 ± 1.3 days following open repair. The difference was statistically significant: Standardized Mean Difference (SMD) = –0.61 [95% CI: –0.86; –0.35]; p < 0.0001, with substantial heterogeneity (I^2 = 96.9%).

These results indicate that patients undergoing robotic repair were discharged on average one day earlier than those who underwent open surgery. The most pronounced difference was reported by Pokala B. (2019) [18] — 1.75 ± 1.62 days (RS) vs. 3.57 ± 4.10 days (OS), and by Rodrigues-Gonçalves V. (2024) [13] — 1.0 ± 0.2 days (RS) vs. 1.7 ± 2.9 days (OS) (Figure 6A).

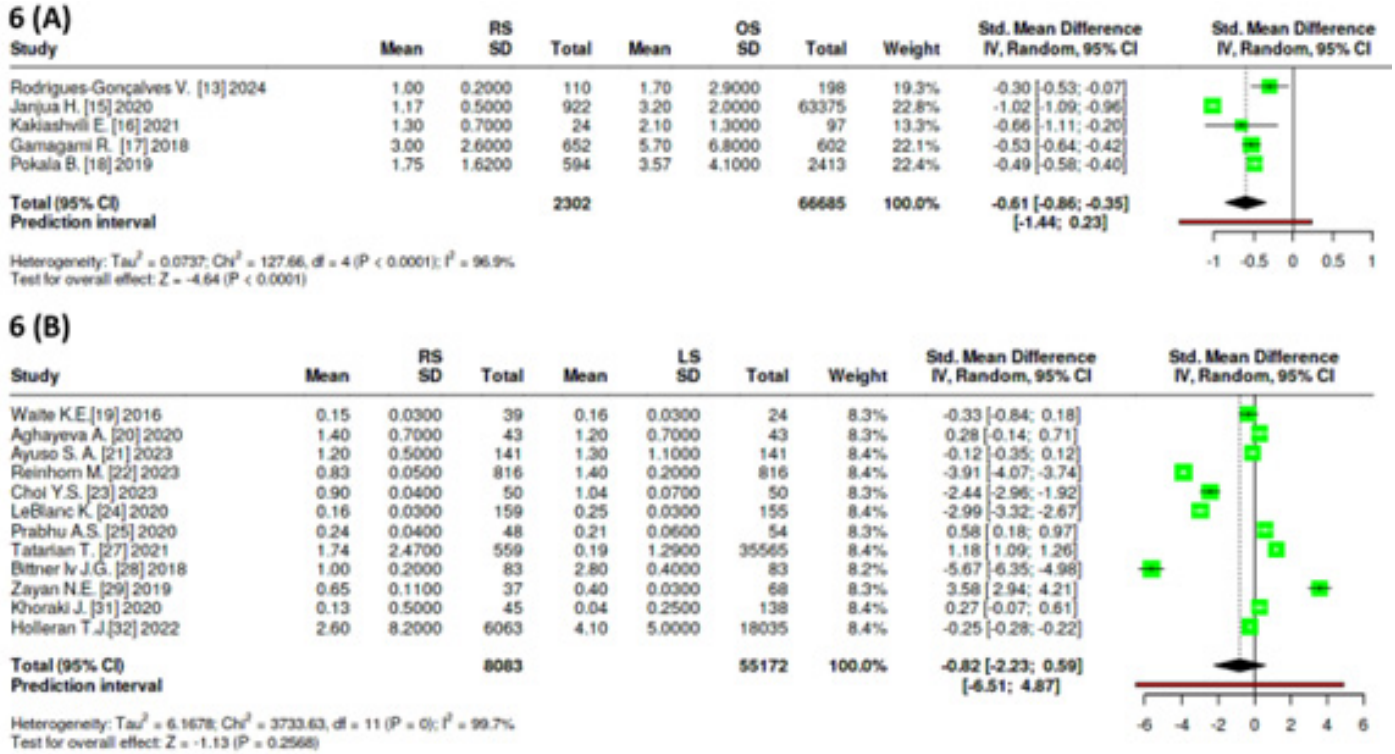


Figure 6 – Mean hospital stay after different hernia repair techniques: (A) — Robotic (RS) vs. Open (OS) surgery; (B) — Robotic (RS) vs. Laparoscopic (LS) surgery

RS — robotic surgery; LS — laparoscopic surgery; OS — open surgery; SMD — standardized mean difference; CI — confidence interval.

RS vs. LS. Analysis of 12 studies (n = 63,934 patients: 8,083 robotic and 55,172 laparoscopic repairs) revealed that the mean hospital stay for robotic procedures was 1.1 ± 0.5 days, compared to 1.4 ± 0.6 days for laparoscopic repairs. The pooled results showed no statistically significant difference between the two methods: SMD = –0.82 [95% CI: –2.23; 0.59]; p = 0.2568, with high heterogeneity (I^2 = 99.7%).

Despite the lack of statistical significance, there was a consistent trend toward a shorter hospital stay in the RS group. For instance, Holleran T.J. (2022) [32] and Ayuso S.A. (2023) [21] reported mean hospital stays of 2.6 ± 8.2 and 1.2 ± 0.5 days after RS, compared with 4.1 ± 5.0 and 1.3 ± 1.1 days after LS, respectively. Similarly, Aghayeva A. (2020) [20] observed comparable results — 1.4 ± 0.7 vs. 1.2 ± 0.7 days (Figure 6B).

RS, LS, OS. Integrated analysis of data from Tables 3 and 4 allowed assessment of mean hospital stay across the main approaches (Table 8).

Table 8

Comparative Mean Hospital Stay Following Different Hernia Repair Techniques (Pooled Meta-Analysis Results)

Technique	Mean Hospital Stay (days)	Range Across Studies (days)	Standardized Mean Difference (SMD)	p-value
Open (OS)	2.1	1.3–5.7	– (reference)	—
Laparoscopic (LS)	1.4	0.9–4.1	–0.82 [–2.23; 0.59]	0.2568
Robotic (RS)	1.1	0.15–3.0	–0.61 [–0.86; –0.35]	< 0.0001

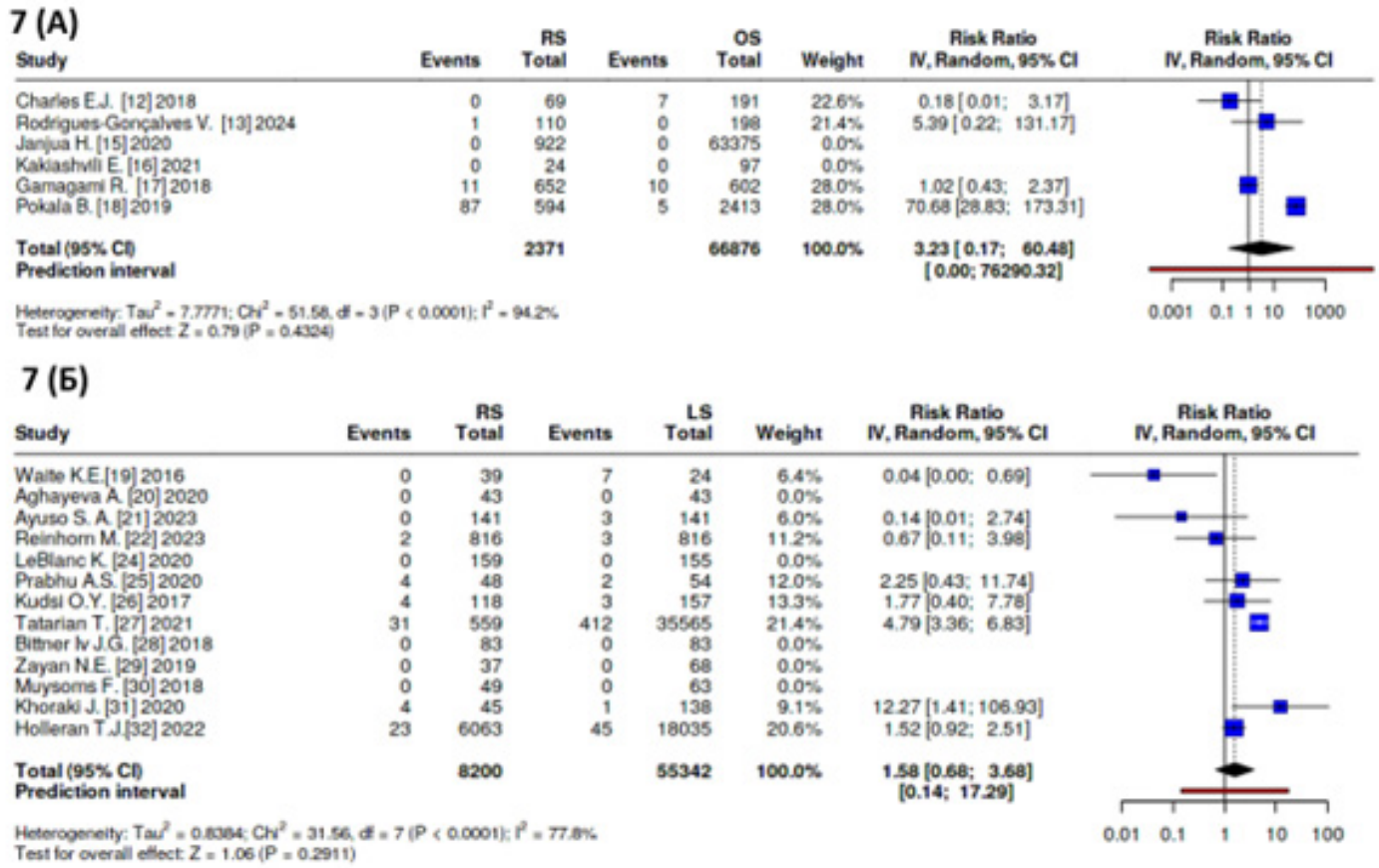
Overall, the mean hospital stay after minimally invasive procedures (robotic and laparoscopic) ranged between 1–1.5 days, compared with approximately 2–3 days after open surgery. These findings confirm that robotic hernioplasty promotes faster recovery, reduced analgesic requirements, and earlier mobilization of patients. The shorter hospitalization period following RS also reflects lower surgical trauma and optimized rehabilitation compared with traditional open techniques.

Results of Comparative and Meta-Analysis of Readmission Rates

RS vs. OS. The meta-analysis included four studies encompassing 69,047 patients (2,371 robotic and 66,676 open repairs). The average readmission rate after robotic surgery was 1.3%, compared with 2.1% following open repair. The pooled risk estimate was OR = 3.23 [95% CI: 0.17–60.48]; p = 0.43, with substantial heterogeneity (I^2 = 94.2%). Although the difference was not statistically significant, there was a consistent trend toward a lower readmission rate following robotic hernioplasty. The largest discrepancy was reported in Charles E.J. (2018) — no readmissions in the RS group versus seven cases in the OS group. Conversely, Pokala B. (2019) [18] reported 87 readmissions after RS and five after OS, a difference explained by the inclusion of patients with complicated hernia forms.

Overall, the data indicate that robotic repair does not increase the risk of readmission compared with the traditional open approach (Figure 7A).

RS vs. LS. The meta-analysis of eight studies (n = 63,752 patients: 8,200 robotic and 55,552 laparoscopic repairs) demonstrated comparable readmission rates, averaging 1.0–1.5% in both groups. The pooled analysis yielded OR = 1.58 [95% CI: 0.68–3.68]; p = 0.29, with moderate heterogeneity



RS — robotic surgery; LS — laparoscopic surgery; OS — open surgery; SMD — standardized mean difference; CI — confidence interval.

Table 9

Comparative Readmission Rates After Different Hernia Repair Techniques (Pooled Meta-Analysis Results)

Technique	Mean Readmission Rate (%)	Range Across Studies (%)	Odds Ratio (OR)	p-value
Open (OS)	2.1	0–7	1.00 (reference)	—
Laparoscopic (LS)	1.4	0–6	1.58 [0.68–3.68]	0.291
Robotic (RS)	1.3	0–5	3.23 [0.17–60.48]	0.432

(I^2 = 77.8%). The highest readmission rates following RS were reported in Tatarian T. (2021) [27] and Khoraki J. (2020) [31], both of which included patients with recurrent and bilateral inguinal hernias. In other studies — such as Ayuso S.A. (2023) [21], Reinhorn M. (2023) [22], and Aghayeva A. (2020) [20] — the rates remained consistently low, ranging from 0 to 1%. Collectively, these findings suggest that robotic hernioplasty does not increase the risk of readmission compared with laparoscopic repair (Figure 7B).

RS, LS, OS. Integration of pooled data revealed the following trend (Table 9).

Discussion

The results of this systematic review and meta-analysis confirmed that the choice of surgical technique for inguinal hernia repair has a significant impact on postoperative recovery, complication rates, and length of hospitalization. The observed increase in pooled odds of recurrence following robotic repair should be interpreted with caution. Despite this statistical finding, absolute recurrence rates remained low, and the result is likely influenced by learning-curve effects, limited follow-up, and small early robotic cohorts.

The analysis revealed that operative time for robotic hernia repair exceeded that of both laparoscopic and open techniques. The mean operative timefor RS was 96 ± 41 minutes, compared to 78 ± 36 minutes for LS and 60 ± 17 minutes for OS. The longer time was primarily attributed to the docking and setup phases of the robotic system, as well as to the precision required for delicate maneuvers in confined anatomical spaces. However, as surgeons gain proficiency, this time difference decreases by 15–25%, which aligns with findings from Muysoms et al. (2018), where the duration of robotic procedures decreased significantly after the first 30–40 cases [30]. Thus, robotic hernioplasty matches laparoscopic repair in both efficacy and safety, and the longer operating time does not negatively affect overall clinical outcomes.

The analysis of postoperative complications demonstrated a clear trend toward reduction when using minimally invasive techniques. The mean complication rates were 7.3% for open, 5.6% for laparoscopic, and 4.1% for robotic repairs. These findings are consistent with international registry data (HerniaSurge, 2018; Huerta et al., 2019) indicating that minimally invasive approaches reduce the risk of surgical site infections and chronic pain by 1.5–2 times [1,14]. The benefits of robotic surgery were particularly evident in recurrent and bilateral hernias, where precise dissection and restoration of the inguinal canal anatomy are crucial. In a multicenter study, Reinhorn et al. (2023) demonstrated that the robotic approach reduced the risk of reoperations by 28% compared with conventional access [22].

A key advantage of the robotic technique is the shorter hospital stay. On average, patients undergoing RS were discharged after 1.1 days, compared with 1.4 days after LS and 2.1 days after OS. These results confirm that robotic hernioplasty allows faster rehabilitation and earlier mobilization, which is especially relevant in the context of Enhanced Recovery After Surgery (ERAS) protocols. Similar findings were reported by Pokala et al. (2019), who observed that the average hospital stay after minimally invasive repair was 40% shorter than after open procedures [18]. Reduced tissue trauma, improved visualization, and more precise mesh placement contribute to this faster recovery trajectory.

Another noteworthy finding concerns readmission rates, which remained low across all techniques and did not exceed 2%. Robotic hernioplasty showed the lowest readmission rate (1.3%) compared with laparoscopic (1.4%) and open (2.1%) repairs. This underscores the reliability and stability of minimally invasive techniques. Comparable results were reported by Holleran et al. (2022), where readmission rates after RS and LS were 1.1% and 1.6%, respectively [32].

From a clinical perspective, robotic surgery achieves an optimal balance between safety, precision, aesthetic outcome, and postoperative comfort. Despite the slightly longer operative time, RS consistently demonstrates superior results in hospital stay duration, recovery speed, and patient satisfaction. Laparoscopic hernioplasty remains a cost-effective and dependable method, particularly in resource-limited settings. Meanwhile, open repair remains justified in cases of contraindications to insufflation or emergency presentations, although its utilization is steadily declining in favor of minimally invasive approaches.

In summary, the findings confirm that robotic hernioplasty is a safe and effective alternative to conventional techniques. Its advantages are most pronounced in complex and bilateral hernias and among patients seeking minimal postoperative pain and early discharge. While operative timeremains longer, the clinical and functional outcomes of RS surpass those of open repair and are fully comparable to laparoscopic surgery. Future studies should focus on long-term recurrence rates, cost-effectiveness, and the influence of the surgical learning curve on clinical outcomes.

Study Limitations. Several limitations should be acknowledged. Most of the included studies had a retrospective design, which increases the risk of selection and reporting bias. Considerable interstudy heterogeneity (I^2 up to 90%) was observed, driven by differences in surgeon experience, patient characteristics, mesh fixation protocols, and complication reporting criteria. Not all studies provided stratified data according to hernia classification (Nyhus, EHS), defect size, or body mass index, limiting the potential for detailed meta-regression analysis.

Additionally, the follow-up duration in several studies was limited to 6–12 months, precluding assessment of long-term recurrence and chronic pain rates. Finally, economic parameters — including costs of consumables, amortization of robotic platforms, and surgeon training — were inconsistently reported, complicating comprehensive cost-effectiveness evaluation.

Scientific Novelty and Practical Significance. This study presents a comprehensive systematic review and meta-analysis providing a direct three-way comparison of open, laparoscopic, and robotic inguinal hernia repair using standardized statistical methods (odds ratios, standardized mean differences, and 95% confidence intervals). In contrast to previous meta-analyses that

predominantly focused on pairwise comparisons between two surgical techniques, the present review integrates aggregated data from more than 66,000 patients, thereby substantially strengthening the level of evidence. The findings offer a unified comparative framework that facilitates evidence-based surgical decision-making and supports individualized selection of the operative approach in clinical practice.

The scientific novelty lies in confirming the equivalence of robotic and laparoscopic approaches in recurrence rates while demonstrating fewer complications and shorter hospital stays with robotic surgery. The practical significance of this research lies in its potential to inform clinical guidelines, patient stratification, and technique selection based on anatomical complexity, surgeon experience, and institutional resources. Moreover, the identified trend toward shorter hospitalization and accelerated recovery supports the expansion of ambulatory (one-day) surgery programs and the implementation of ERAS protocols in inguinal hernia management.

Conclusion

1. This systematic review and meta-analysis demonstrate that laparoscopic and robotic inguinal hernioplasty are associated with favorable perioperative outcomes, including lower postoperative complication rates and shorter hospital stay, compared with open Lichtenstein repair.

2. Robotic hernioplasty is characterized by a longer operative time relative to laparoscopic and open techniques, which is largely attributable to learning-curve effects. Available evidence suggests that operative duration decreases with increasing surgeon experience without compromising clinical outcomes.

3. Absolute recurrence rates across all surgical techniques remain low; however, pooled analysis indicates higher odds of recurrence following robotic repair compared with open surgery. This finding should be interpreted cautiously, given the influence of early robotic series, limited follow-up duration, and surgeon experience.

4. Minimally invasive approaches significantly reduce hospital stay, postoperative pain, and early recovery time, thereby supporting their advantages in terms of patient rehabilitation and short-term quality of life.

5. Robotic-assisted hernioplasty may offer technical advantages in selected complex cases, such as bilateral or recurrent inguinal hernias, owing to enhanced visualization and instrument precision; however, robust comparative evidence remains limited.

6. Overall, laparoscopic hernioplasty represents a well-established and effective minimally invasive alternative to open repair, while robotic hernioplasty remains a promising but evolving technique requiring further validation.

7. Further large-scale randomized controlled trials with long-term follow-up and standardized outcome measures are necessary to clarify the role of robotic hernioplasty, particularly with respect to recurrence, cost-effectiveness, and patient selection.

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Ethical Aspects of Using CRISPR-Cas9 in Medicine in Kazakhstan and Worldwide

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ABSTRACT

CRISPR-Cas9 is a new emerging genome editing tool, that gives hope as a cure to various diseases, including cancer and hereditary diseases. Although its accuracy in editing DNA is fascinating, recent breakthroughs in this field raise crucial ethical and legal issues. Usage of CRISPR-Cas9 raises concerns pertaining to safety, availability, religious and societal impact. Nowadays, some of the high-income countries have developed regulatory frameworks to address these concerns, however there is still a lack of global concrete regulations for all nations. In Kazakhstan, even though genome editing technology is used mostly in research, we are in demand of developing bioethical principles based on international practices. The regulatory framework would ensure a responsible approach in using this tool in medicine and research. In this review, we explored international ethical practices and examined the current situation in Kazakhstan in implementing genome editing technology. CRISPR-Cas9 is a new emerging genome editing tool, that gives hope as a cure to various diseases, including cancer and hereditary diseases. Although its accuracy in editing DNA is fascinating, recent breakthroughs in this field raise crucial ethical and legal issues. Usage of CRISPR-Cas9 raises concerns pertaining to safety, availability, religious and societal impact. Nowadays, some of the high-income countries have developed regulatory frameworks to address these concerns, however there is still a lack of global concrete regulations for all nations. In Kazakhstan, even though genome editing technology is used mostly in research, we are in demand of developing bioethical principles based on international practices. The regulatory framework would ensure a responsible approach in using this tool in medicine and research. In this review, we explored international ethical practices and examined the current situation in Kazakhstan in implementing genome editing technology.

Keywords: CRISPR-Cas systems; Genome editing; Bioethics; Human genome; Kazakhstan.

Introduction

There is significant progress in using the CRISPR-Cas9 genome editing system, as a solution to many diseases. Precise modifications in human genome allowed to eradicate mutated parts of DNA and give hope for the treatment of genetic diseases, cancer and improved disease prevention. In Kazakhstan, their importance is shaped by the country's unique socio-political, regulatory, and scientific context.

One of the main ethical concerns with genome editing technology lies on the difference between therapeutic use and genetic improvements. The ethical aspects of using CRISPR to cure serious hereditary diseases are widely accepted. However, its potential impact on non-medical traits, as intelligence, physical performance raises complicated bioethical issues. The United States (US) and countries of the European Union (EU), prohibited usage of human germline genetic enhancements, taking into account its unknown future risks. The use of CRISPR-Cas9 was authorized in United Kingdom (February 2016) to edit human embryos, but only for research purposes and under strict regulations [1]. The research focused on Octamer-binding transcription factor 4 (OCT4) gene, which is considered to be one of the key players during embryo development. The edited embryos were not further implanted to woman and

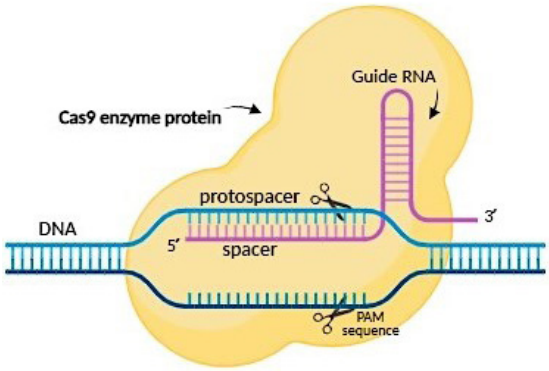

Figure 1 – Illustration of CRISPR-Cas9 Genome Editing

were destroyed after a week of cultivation. Results of the experiment helped to understand underlying mechanisms of embryo development, which could be used in the future during in vitro fertilization (IVF) and for the treatment of infertility [2].

CRISPR-Cas9 genome editing is the process of cutting specific sites of DNA by Cas9 enzyme protein and guide RNA. This complex recognizes specific regions of DNA. To find the target DNA region, photospacer adjacent motif (PAM) is required. PAM is located next to the target, and without PAM, Cas9 can`t cut (Figure 1.) [2]. Once the region is cut, creating double-stranded break, cell can either repair or introduce a specific genetic alteration.

The situation is different with somatic cells, since genetic modification of somatic cells does not result in the transmission of changes to offspring and ethically accepted. This breakthrough gave great hope to cure diseases, but at the same time raised concerns regarding the application of technology for non-therapeutic purposes i.e. to enhance athlete's performance. Also, it has potential to influence in violating criminal justice where gene editing could alter the risk of violent behavior [3]. Similar cases urgently push to create strict ethical regulations to avoid misuse of the technology.

Another important concern is related to modifying gene pool for the prevention of the inherited diseases as Huntington`s disease or sickle cell anemia, where target genes could be eradicated from germ cells. However, it raises debates about the patient consent and responsibility for future potential consequences [4,5].

The lack of clear regulations heightens the risk of uncontrolled experiments or uncertainties in genome editing research. In Kazakhstan, the lack of a regulatory framework in genome editing technology, raises the risk of uncontrolled experiments. The fast-evolving genome editing tool demands thorough and strict regulations, taking into account local perception and international standards.

This paper aims to compare and elaborate on the current genome editing technology status and ethical regulations in Kazakhstan and worldwide.

Global Ethical Concerns in Medicine

Human Germline Editing

There must be a clear distinction between the ethical aspects of using CRISPR on somatic cells and germline cells. Modified gene in somatic cells impacts only the treated individual, whereas germline gene alteration could potentially affect multiple generations [6]. Proponents of germline editing argue on permanently removing inherited diseases, whereas opposers bring forward the potential risks of unpredictable future outcomes [7]. Due to the possible long-term consequences, the World Health Organisation (WHO) and other international agencies recommended a temporary pause on germline editing [8].

Equity and Accessibility

Potentially life-saving CRISPR gene-editing technologies should be available to everyone, including middle- and low-income countries. The high cost of treatment raises concerns of fair access and equity. Nowadays, advanced medical therapies are mostly available for high-income countries or wealthier individuals, which makes existing global health disparities worse [9]. This kind of disparities could lead to genetic divide of whole population into two categories, one who has protection with superior biological traits and the vulnerable group. Therefore, it is essential to create regulations ensuring equal access.

Dual-Use Concern

Alongside with rapid development of genome editing technology in medicine and research, dual-use concerns arise. Technology potential can be used for creating biological weapons or pathogens with resistance to existing treatments. The United Nations are highly concerned about these risks [10]. Experts are also alarmed with the activities of the Defense Advanced Research Projects Agency`s (DARPA) "Insect Allies" program, initially created to modify crops. There is a risk of creating genetically modified biological agents, which could pose serious biological threats [11].

There is some ongoing research on boosting soldier performance, i.e. in China boosting soldier endurance are being discussed [12], while US is looking to defend against chemical weapons [13]. Potentially this technology could be used for lessening sleep needs, tolerate pain and lowering stress levels. Although these developments are seeming to be good, there are still vital bioethical concerns and human right issues to be considered.

Case Study: CRISPR Babies in China

The first incident of using CRISPR technology on human was announced in November 2018 by scientist He Jiankui. Researcher modified C-C chemokine receptor type 5 (CCR5) gene to make offspring resistant to Human Deficiency Virus (HIV). Although genetically modified twins were born healthy, absence of prior ethical approval and regulatory permission sparked worldwide condemnation [14]. As a result, it caused unverified genetic changes with unknown long-term effects [15].

Although He Jiankui claimed that the gene editing was "successful" and that the children were now fully or partially immune to HIV, many scientists and experts pointed out that there is no proof of this [16].

After his release from prison in April 2022, He Jiankui established a new laboratory in Beijing. He aims to develop affordable gene therapy for rare genetic diseases, such as Duchenne muscular dystrophy. [17]. In addition, he plans to establish a non-profit research organization called the Beijing Institute for Rare Disease Research.

The scientific community widely criticized He Jiankui for breaching bioethical guidelines and medical ethics, as well as for performing unauthorized experiments on humans. This incident pushed China`s government to create strict control over CRISPR usage and called for global ethical oversight in genome editing.

Table 1 Comparative Analysis of CRISPR-Cas9 Applications by Country

Country	Start Year	Application Focus	Research Methodologies	Regulatory & Ethical Considerations	Clinical & Experimental Outcomes
China	**2016**	Oncology, embryo editing	In vivo and ex vivo gene editing using CRISPR-Cas9 in human T-cells	Controversial embryo editing in 2018 led to stricter regulations	Ongoing trials in oncology and genetic disorders
USA	2017	Genetic blood disorders, cancer	Stem-cell-based CRISPR therapy; CRISPR screens for gene function	FDA-regulated clinical trials; ethical concerns over germline editing	First FDA-approved CRISPR therapy (2023, Exa-cel)
UK	2016	Fundamental CRISPR research, embryo studies	CRISPR applied to early-stage embryonic development	Strict adherence to bioethical standards	Permitted CRISPR use in non-implantable embryos
EU	2017	Genetic and rare diseases	Genome-wide CRISPR libraries for gene function analysis	Harmonized regulation under EMA; high ethical oversight	Promising results in treating cystic fibrosis and hematologic conditions
Japan	2019	Ophthalmology, regenerative medicine	CRISPR editing in stem-cell-derived retinal cells	Limited clinical applications due to strict gene-editing policies	Ongoing studies for hereditary blindness treatment
Russia	2019	Embryonic gene editing, rare diseases	Focus on theoretical and in vitro models	Minimal government investment; ethical debates on human gene editing	No active clinical trials; preliminary research stage

CRISPR – Clustered Regularly Interspaced Short Palindromic Repeats; EMA – European Medicines Agency; FDA – Food and Drug Administration.

Ethical Aspects in Kazakhstan

CRISPR technology is also advancing in Kazakhstan, especially in medicine and agriculture. CRISPR-Cas13 was used by scientists from L.N. Gumilyov Eurasian National University. The study aimed to protect plants from viruses by targeting viral DNA sequences of viruses, i.e. tombusviruses. Results showed increased immunity and resistance of plants to viruses.

Bakhytzhan Alzhanuly and his colleagues from the Institute of Molecular Biology and Biochemistry demonstrated significant results in curing type 1 diabetes [18]. The research focused on altering genes of pancreatic cells, that could produce insulin and self-regulate its levels. This breakthrough can provide alternative treatment by replacing traditional insulin injections. The main purpose of this study is to transplant genetically modified beta cells into patients' pancreases, offering a potential long-term therapy [18]. Although there is no definitive cure for type 1 diabetes [19], injecting genetically modified cells to human may potentially raise bioethical issues, based on unknown long-term effects on the body.

Currently there is a lack of specific laws regulating usage of CRISPR in Kazakhstan. As international practices show, there are high risk of repetition of similar cases related to germline editing, dual-use nature. Therefore, there is an urgent need of creating certain governmental bodies who will control bioethical aspects of CRISPR. Currently Kazakhstan has regulations pertaining biomedical research, particularly Articles 227 and 228, which outline acceptable participant types, informed consent procedures, bioethical oversight, and insurance requirements [20]. Under these laws it is permitted to do research on human, animals, biological samples, but not on cloning or creating embryo. Also, participation of minor groups as pregnant women, military personnel and prisoners are allowed under strict conditions. Additionally, interventional studies require participants to have health insurance.

Based on existing international regulations, Kazakhstan should implement concrete regulations on genome editing oversight in short term run. These regulatory frameworks should cover religious and cultural backgrounds of locals, through organizing public and interfaith hearings. In firsthand National Council of Bioethics should be established, which will be updating regulations according to scientific advancements worldwide and will oversee ethical governance. This governmental body will be responsible for accreditation of institutes who use CRISPR. Moreover, specialized bioethics courses should be introduced in medical and biotechnological universities to train future professionals, organize audits.

Cultural and Religious Considerations

One of the main steps in creating fully functional regulations, lies on increasing public`s limited understanding of CRISPR and its potential consequences. Law aligned with public values demands analysis of religious beliefs, traditions, fostering open dialogues among scientists, policymakers and public representatives. Kazakhstan has multinational and multicultural country. Therefore, it is crucial to consider every tradition and religion views on CRISPR. This can be achieved by surveys engaging religious and cultural leaders.

The system with great consideration of public value will shape society`s cautious approach to such innovations, especially in fast growing genome editing technology [21].

Official religion of Kazakhstan is Islam, and most of the population follow the regulations of this religious belief. According to teachings of Islam, any interference to human nature, especially effecting future generations permitted if that must be done in life-saving conditions, justified and has solid medical and ethical reasons [22]. Obviously, there are other religions practiced as well, every religion has their own pros and cons, thus it is very important that ethical regulatory framework take these aspects into consideration while creating the law.

Conclusion

The ethical aspects of gene editing technology are complex and demands careful approach. As technology tends to grow fast, it is crucial to create comprehensive ethical guidelines,

in order to avoid past internationally evidenced story. This is especially important for germline editing. Kazakhstan needs to create a well-balanced, socially accepted ethical regulations to maximize benefits of CRISPR-based therapies and promote fair access to life saving treatments.

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Young Patient Presenting with ST-elevation Myocardial Infarction (STEMI) Following Alcohol Mixed with Energy Drink (AMED)

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ABSTRACT

In young myocardial infarction patients (<45), causes such as energy drinks, alcohol, cocaine, and anabolic steroids are added to the etiology, unlike in older myocardial infarction patients. Our case is a 19-year-old male who presented to the emergency department with ST-elevation MI after consuming alcohol mixed with an energy drink (AMED). Coronary angiography revealed a 99% thrombotic lesion in the distal Cx, which was thin in structure, in a patient whose coronary artery structure was not atherosclerotic. Due to the thinness of the vessel structure, medical treatment was initially planned for the patient.

Keywords: alcohol, energy drink, AMED, STEMI, myocardial infarction.

Introduction

Despite advances in technology and medications, acute myocardial infarction remains one of the leading causes of mortality [1]. There are long-established risk factors for acute myocardial infarction, such as hypertension, diabetes mellitus, hyperlipidemia, being male, and smoking [1,2]. However, the risk factors for myocardial infarction observed in young patients under the age of 45 are changing [2,3]. Risk factors such as smoking, male gender, obesity, diabetes mellitus, familial combined hyperlipidemia, cocaine and cannabis use, and androgenic anabolic steroid use are risk factors that should be investigated in patients with young myocardial infarction [2,3,4,5].

In this case presentation, we will present a case with a diagnosis of acute myocardial infarction whose etiology differs from the usual risk factors.

Case Report

A 19-year-old male with no known medical history presented to the emergency department complaining of chest pain after consuming energy drinks and alcohol together for two consecutive days (3 x 250 ml on the first day, 2 x 250 ml on the second day; each 250 ml energy drink contained 80 mg of caffeine). The patient had no known history of chronic disease, exercised regularly for 90 minutes per week, and had a BMI of 22.86 kg/m2. The ECG showed ST elevation in the D1, AVL, V5, and V6 leads (Figure 1: The Patient's ECG). The patient was taken to the coronary angiography catheterization unit for emergency angiography. Angiography showed normal LMCA, normal LAD, a 99% thrombotic lesion in the distal Cx, and normal RCA. The distal Cx was observed to be narrow (<2 mm), and 5000 IU of intracoronary heparin and a 100 µg bolus of glyceryl trinitrate were administered. No increase in distal vessel diameter was observed, and TIMI II flow was seen distally, leading to the decision to proceed with

medical treatment. The patient's troponin I value was 5574.6 ng/L (0-20 ng/L). The echocardiogram showed an ejection fraction of 60% with no segmental motion defect. The patient was started on enoxaparin 6000 IU twice daily, acetylsalicylic acid 300 mg loading dose followed by 100 mg once daily, clopidogrel 600 mg loading dose followed by 75 mg once daily, atorvastatin 40 mg once daily, and metoprolol 50 mg tablet once daily. A toxicological screening (for synthetic cannabinoids, amphetamines, barbiturates, benzodiazepines, buprenorphine, cannabinoids, cocaine, and opiates) was performed on the patient's urine, and no toxic substances were detected. The patient was consulted with the internal medicine and rheumatology departments. Following blood tests, hyperlipidemia, diabetes mellitus, rheumatological diseases, and vasculitis were excluded from the etiology. Energy drinks and alcoholic cocktails were identified as the most likely cause of young heart attacks. During a total of 4 days of follow-up, including 2 days in the intensive care unit and 2 days in the inpatient department, the patient, who had no chest pain, was discharged with a prescription for 100 mg of acetylsalicylic acid once daily, 75 mg of clopidogrel once daily, 40 mg of atorvastatin once daily, and 50 mg of metoprolol once daily. The patient's follow-ups at 1 month and 2 months were performed; no ST elevation was observed on the ECG, and LV systolic function was normal on echocardiography. Due to intermittent palpitations, a 24-hour rhythm Holter monitoring was performed. Sinus tachycardia was observed during the hours when symptoms were reported, and no arrhythmia was detected. The metoprolol dose was increased from 50 mg/g to 100 mg/g. There was no chest pain during follow-ups.

Discussion

Energy drinks, discovered approximately 100 years ago and becoming increasingly popular over the last 10 years, contain high doses of caffeine, guarana, and other stimulants [6,7]. Energy drinks can be consumed on their own or in combination with alcohol. Alcohol mixed with energy drinks (AMED) is a form of consumption more commonly seen among young people because energy drinks reduce the intoxicating effects of alcohol. A study conducted in the USA found that AMED consumption among students and young people ranged from 8.1% to 64.7% [8].

A study conducted in healthy young volunteers on energy drinks showed that after consuming 1 can (250 mL), platelet aggregation increased acutely and endothelial function decreased [9]. Another study of healthy adults found that consuming energy drinks increased heart rate and systolic blood pressure [10].

When we evaluate the cardiovascular effects of alcohol and energy drinks together, alcohol can cause myocardial infarction by inducing vasospasm, inhibiting the fibrinolytic system, and increasing thrombus formation, while energy drinks can cause myocardial infarction by increasing catecholamines, increasing intracellular cyclic adenosine monophosphate, and reducing angiotensin II [6,11,12, 13].

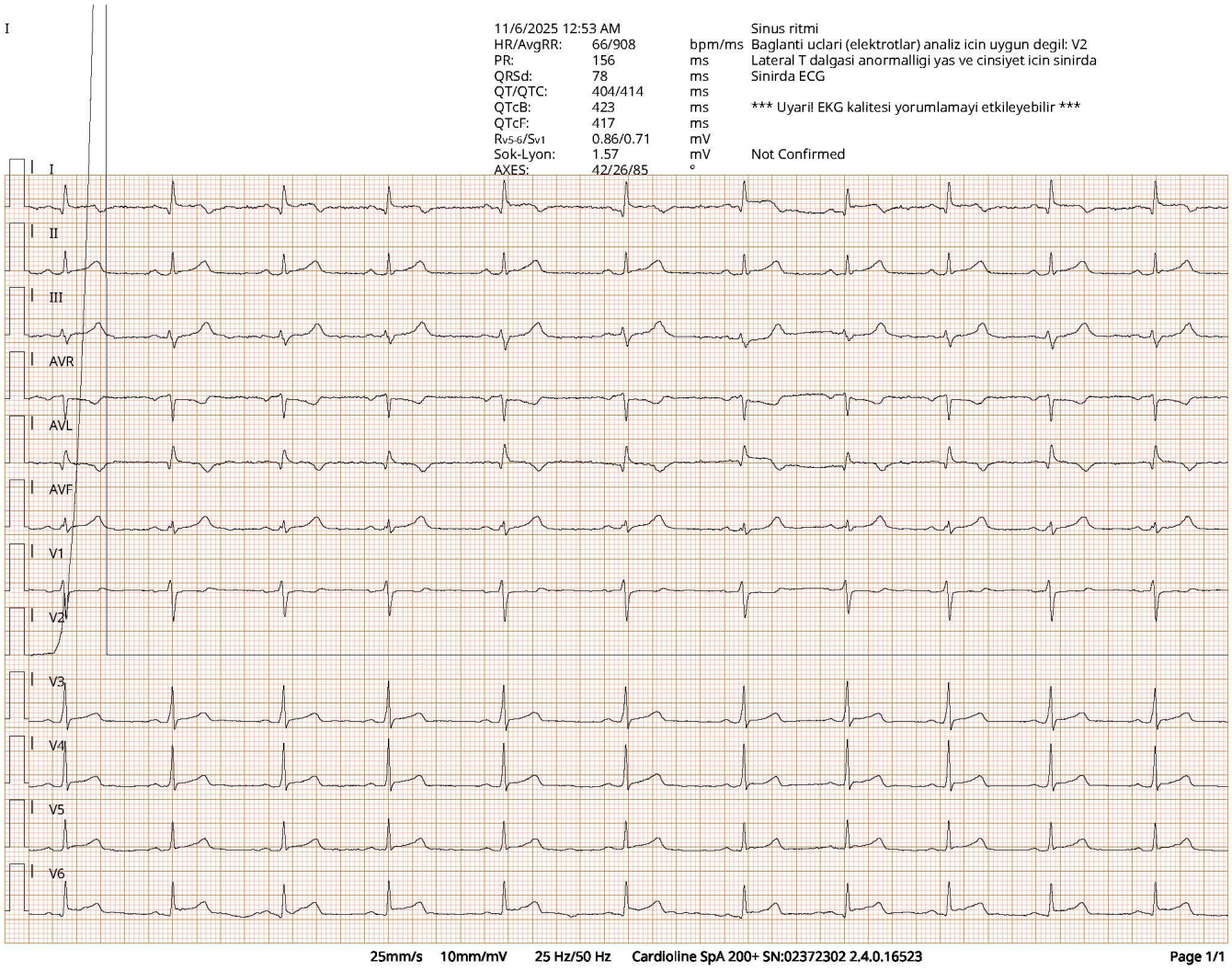

Figure 1 – The patient's ECG (ST elevation in D1, AVL, V5, and V6 leads)

In our patient, the presence of a lesion only in the distal Cx and the normal structure of the other coronary arteries, along with the absence of additional risk factors, leads us to consider myocardial infarction following the consumption of alcohol mixed with energy drinks as the most likely cause.

The limiting factor in our case is that we did not have IVUS access during coronary angiography and could not identify any individual or familial risk factors in our detailed etiological investigation, apart from the combined use of energy drinks and alcohol.

Conclusion

In conclusion, it is useful to be cautious when diagnosing acute cardiovascular disease in young patients. It would be beneficial to provide information about alcohol mixed with energy drinks, particularly in schools, and to offer education on measures to reduce their consumption.

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Managing Seizure Control and Neuropsychiatric Impact: A Case of Medication Compliance

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ABSTRACT

Epilepsy is characterized by a long-standing tendency to generate unprovoked seizures, accompanied by a range of neurobiological, cognitive, psychological, and social consequences. The condition is slightly more prevalent in males, particularly among the elderly, due to a higher incidence of strokes, neurological disorders, and tumors in this group. Focal seizures are more commonly observed than generalized ones across both pediatric and adult populations. When assessed in terms of seizure control, many individuals with epilepsy demonstrate a favorable prognosis. However, in low- and middle-income countries (LMICs), a significant treatment gap persists due to factors such as misdiagnosis, inadequate access to medications, acute symptomatic seizures, and increased early mortality. Research indicates that nearly half of epilepsy patients eventually achieve sustained seizure remission. This report illustrates the case of 22-year-old young man with a history of epilepsy and the complex interplay between medication adherence, epilepsy management and the broader impacts on his health.

Keywords: Epilepsy, strokes, mortality, neurobiological, focal seizures.

Introduction

Epilepsy is among the most common neurological disorders worldwide, affecting individuals across all age groups, ethnicities, and geographic regions, with an estimated global prevalence of 50 million people [1]. It is characterized by recurrent unprovoked seizures caused by abnormal neuronal discharges in the brain [2]. The diagnosis is established after two or more unprovoked seizures occurring more than 24 hours apart in the absence of acute provocation [3].

Beyond the neurological manifestations, epilepsy is often associated with neuropsychiatric comorbidities such as depression, anxiety, and irritability, which can significantly impair quality of life and treatment adherence [4]. These comorbidities may arise due to overlapping neurobiological mechanisms or as adverse effects of long-term AED use [5].

Medication non-adherence remains a major challenge in epilepsy management and is responsible for increased seizure recurrence, emergency admissions, and poor psychosocial outcomes [6]. Despite effective pharmacotherapies, up to 30% of patients continue to experience breakthrough seizures due to poor compliance or drug resistance [7].

Rationale and Aim: The present case highlights the clinical consequences of poor medication adherence in epilepsy and the subsequent neuropsychiatric impact. It also illustrates how careful drug selection and patient education can improve seizure control and overall mental well-being.

Case Presentation

A 22-year-old male was diagnosed with epilepsy at age 10 after his first generalized tonic–clonic seizure (GTCS) occurred in a school classroom. His past history revealed a violent altercation in which he was stabbed in the back with a pencil, leading to localized

calp swelling that was treated at home without medical intervention.

In the subsequent weeks, the patient exhibited episodes of unresponsiveness, blank staring, and sudden behavioural arrest. Neurological evaluation confirmed epilepsy, and valproic acid (Valparin 200 mg twice daily) was initiated. The patient achieved partial seizure control over the next few years.

However, from adolescence onward, he exhibited poor adherence to medication, frequently missing doses for days or weeks. This resulted in multiple seizure relapses characterized by body stiffening, loss of consciousness, and postictal confusion. He was hospitalized on several occasions for uncontrolled seizures.

During follow-up, the patient reported mood swings, irritability, and crying spells. These symptoms coincided with periods of inconsistent valproic acid use. Neurological reassessment revealed no focal deficits. His brain MRI (non-contrast) showed no structural abnormalities (Figure 1), and EEG demonstrated bilateral rhythmic alpha and theta activity consistent with ongoing epileptiform discharges. The electroencephalogram (EEG) demonstrates abnormal sharp-wave discharges predominantly over the right frontal and temporal regions, consistent with focal epileptiform activity.

Table 1 Timeline of Clinical Course

Age	Event	Intervention	Outcome
10 years	First GTCS after head trauma	Diagnosed with epilepsy; started on valproic acid	Partial control
10–15 years	Irregular medication intake	Recurrent seizures	Poor control
16–21 years	Persistent non-adherence, mood changes	Counselling, valproic acid continued	Limited improvement
21 years	Neuropsychiatric symptoms worsen	Lamotrigine added, levetiracetam tapered	Stable mood, seizure-free
22 years (current)	Regular follow-ups, adherence maintained	Continued lamotrigine therapy	Sustained remission

To address mood instability and inadequate seizure control, the treatment plan was modified. Lamotrigine 25 mg/day was introduced and titrated to 100 mg/day over six weeks while valproic acid was gradually reduced. Levetiracetam 500 mg twice daily, which had been added earlier, was tapered and discontinued due to irritability. Over the next 12 months, the patient remained seizure-free, with improved mood and better social functioning. Regular counselling sessions were conducted to reinforce medication adherence and sleep hygiene.

Discussion

Epilepsy requires continuous pharmacological management to prevent recurrent seizures and associated neuropsychiatric complications. Non-adherence to AEDs is estimated to occur in 30–50% of patients and remains one of the strongest predictors of seizure relapse and hospitalization [6,8].

In this case, the patient's intermittent medication compliance directly contributed to seizure recurrence and mood disturbances. Valproic acid, though effective, is known to cause cognitive and emotional side effects in some individuals [9]. The switch to lamotrigine not only stabilized mood but also maintained seizure control, aligning with evidence supporting its favourable psychiatric profile [10].

Levetiracetam, which had been part of the treatment regimen, is frequently associated with irritability and aggression [11]; its gradual discontinuation improved the patient's behaviour. This underscores the need for individualized AED selection based on both neurological and psychological tolerability.

The persistence of EEG abnormalities despite clinical improvement highlights that subclinical epileptogenic activity may continue even during remission [12]. Hence, routine EEG monitoring and patient counselling remain vital components of long-term epilepsy management.

Overall, this case reinforces the need for a multidisciplinary approach encompassing neurologists, psychiatrists, and psychologists to ensure comprehensive care. Patient and caregiver education on the importance of regular medication intake, early recognition of mood changes, and adherence counselling can prevent relapse and improve quality of life.

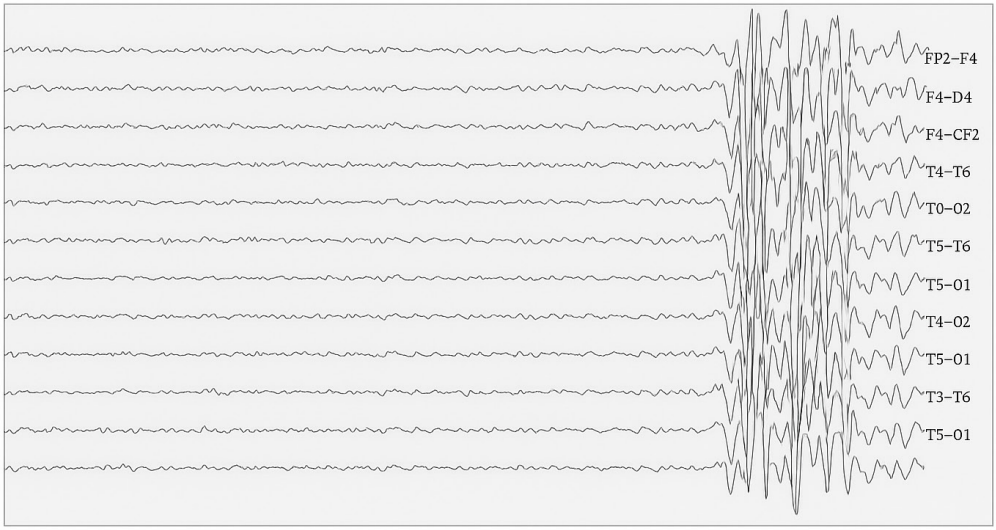

Figure 1 – EEG Recording Showing Epileptiform Activity

Conclusions

This case demonstrates that consistent medication adherence and individualized AED management are crucial for achieving seizure control and psychological stability in epilepsy. Lamotrigine proved effective both for seizure suppression and mood stabilization, while structured follow-up and counselling promoted long-term compliance. A holistic, patient-centred approach remains key to optimizing clinical outcomes in epilepsy care.

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