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A novel benzamide derivative SY-15 inhibits multiple myeloma cell proliferation

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■ MAIN POINTS

- The SY-15 molecule demonstrated significant cytotoxic effects in multiple myeloma cell lines, with the lowest viability observed in the RPMI 8226 cell line, while showing no notable toxicity in normal fibroblast (L929) cells.
- The molecule exhibits high selectivity and a low toxicity profile, making it a strong candidate for patients with MM who have developed resistance to existing therapies.
- Unlike classical benzamides, SY-15 may also modulate alternative apoptotic pathways, which highlights its potential for novel therapeutic combinations, particularly in drug-resistant cell lines.

■ ABSTRACT

Aim: Over the past two decades, the natural course of multiple myeloma (MM) has changed significantly, primarily due to the emergence of novel therapeutic agents targeting the bone marrow microenvironment (BMM). Despite these advancements, the underlying mechanisms of drug resistance remain largely unclear. In this study, the effects of a novel benzamide derivative, SY-15, on MM cell lines were investigated, and the findings suggest that this molecule could be a promising anticancer drug candidate, warranting further research.

Materials and Methods: Multiple myeloma (MM) cell lines (MM1S, U266, H929, RPMI8226) were cultured, and the effects of various concentrations of a novel benzamide derivative on cell viability were evaluated using the MTT assay.

Results: The anticancer activity of the SY-15 molecule was evaluated in multiple myeloma (MM) cell lines following 72 hours of treatment, and for comparison, in the L929 normal fibroblast cell line. A statistically significant difference in cell viability percentages was observed among the five cell lines ($p < 0.001$). The median cell viability percentage was 93.6247 for the L929 cell line, 44.4110 for the MM1S cell line, 22.4655 for H929, 31.7180 for U266, and the lowest median value was recorded in the RPMI 8226 cell line at 13.0931. Notably, SY-15 did not exhibit significant cytotoxicity in L929 fibroblast cells.

Conclusion: SY-15 has the potential to be an effective anticancer agent with high selectivity and low toxicity for the treatment of MM. It may offer a novel therapeutic option, particularly for patients who have developed resistance to other MM drugs.

Keywords: Multiple myeloma, SY-15 molecule, Anticancer drug

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■ INTRODUCTION

Multiple myeloma (MM) is a malignancy made up of B-lymphocytes, and it originates in the bone marrow. They usually have a poor prognosis. It leads to bone marrow suppression, destructive bone lesions, renal dysfunction, immunosuppression, and usually, death is imminent [1]. MM accounts for approximately 1.3% of all cancer diagnoses and 10% of all hematological malignancies. It is the second most common and one of the most lethal hematological cancers. There-

fore, MM continues to be one of the most extensively studied cancer types [2].

According to data from the American Cancer Society, approximately 36,110 new cases of multiple myeloma are expected to be diagnosed in the United States by 2025. Of these, 20,030 will occur in men and 16,080 in women. Additionally, around 12,030 deaths are anticipated (6,540 men and 5,490 women). The lifetime risk of developing MM in the U.S. is less than 1%, with an estimated risk of 1 in 103 for men and 1

in 131 for women. However, individual risk varies depending on personal risk factors [3].

MM is a heterogeneous disease, including progression from asymptomatic precursor stages to active symptomatic myeloma. Almost all MM patients progress from monoclonal gammopathy of undetermined significance (MGUS), an asymptomatic pre-malignant stage of the disease. MGUS is present in approximately 5% of individuals over the age of 50 and is nearly twice as common in Black individuals compared to Caucasians. MGUS carries an annual risk of approximately 1% for progression to MM or related malignancies. Since MGUS is asymptomatic, over 50% of individuals diagnosed with the condition may unknowingly carry it for more than a decade before clinical diagnosis.

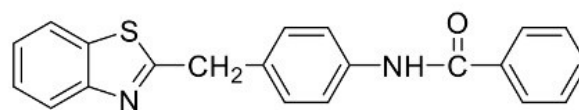
In a significant portion of patients, smoldering multiple myeloma (SMM), which is considered a clinically intermediate stage, can be identified. SMM is present in about 0.5% of the general population over the age of 40 and progresses to MM at an annual rate of 10% in the first five years, 3% in the following five years, and 1.5% per year thereafter. The rate of progression is associated with disease burden and underlying cytogenetic abnormalities; t(4;14) translocation, del(17p) deletion, and gain(1q) are considered significant risk indicators for progression from MGUS or SMM to MM [4].

Multiple myeloma (MM) is characterized by an increasing number of abnormal plasma cells. These cells produce monoclonal proteins (M-proteins), which are detectable in the blood or urine and are key in diagnosing and monitoring the disease. Beyond their diagnostic use, these abnormal proteins can cause serious complications like kidney damage and bone lesions. This highlights the need for early diagnosis and personalized treatment. In about 20% of cases, the disease spreads from the bone marrow to other organs and soft tissues, a condition known as extramedullary disease (EMD). EMD is an aggressive form of MM that complicates treatment and disease management [5].

High-resolution sequencing techniques have advanced our understanding of clonal evolution in multiple myeloma, revealing subclonal diversity and the disease's dynamic nature [6,7,8]. As knowledge of MM pathogenesis and the bone marrow microenvironment improves, various adjunctive cell therapies and new drugs have been developed. Despite recent therapeutic progress, resistance to anticancer drugs remains a major challenge in MM treatment [9]. Research efforts aim to better understand the pathways and protein expressions involved in drug resistance and to develop new therapeutic strategies. Significant progress has been achieved through the sequential or combined use of proteasome inhibitors (PIs), immunomodulatory drugs (IMiDs), monoclonal antibodies (mAbs), and autologous transplantation following high-dose therapy. However, MM is still characterized as a disease that is difficult to control, with alternating periods of remission and relapse/progression, ultimately leading to drug-resistant disease [10].

Recent studies have highlighted that benzamide derivatives induce apoptosis and reduce cell proliferation in various cancer cell lines. Benzamide derivatives are simpler chemical compounds consisting of a phenyl ring directly attached to an amide group, with various substituent groups on the phenyl ring and amide nitrogen [11]. These compounds exhibit notable antibacterial, antifungal, anticancer, and antiallergic activities and have become one of the commonly used intermediates in the synthesis of aromatic ligands [12]. They are known to be more effective against specific HDACs rather than all HDAC classes. In particular, they were shown to strongly inhibit Class I HDACs, including HDAC1, HDAC2, and HDAC3. Benzamides have been reported to induce P21WAF1 expression, cause cell cycle arrest, activate numerous pro-apoptotic genes, and exert cytotoxic effects on various cancer cell types even at very low doses. Some types of benzamides have also been found to inhibit breast cancer, similar to SAHA (suberoylanilide hydroxamic acid). Although natural products of this HDAC inhibitor family have not yet been identified, such drugs have shown promising results in clinical studies on breast cancer and lymphomas [13].

The SY-15 molecule (Figure 1) is a novel benzamide derivative first synthesized by Yilmaz et al. (2013) at Ankara University [14]. This compound was developed as a potential antitumor agent. Structurally, SY-15 features a benzamide core linked to a benzothiazole moiety through a phenyl group. The inclusion of the benzothiazole ring is particularly significant, as it may enhance the molecule's binding affinity to biological targets. This enhancement could occur through hydrophobic interactions, pi-pi stacking, and potential hydrogen bonding with amino acid residues in target proteins. In this study, we investigated the effects of the unique small molecule SY-15 on multiple myeloma (MM) cell lines, including MM1S, U266, H929, and RPMI 8226. Preliminary studies have shown that SY-15 is effective against various types of cancer, suggesting that it may possess significant anticancer activity for the treatment of multiple myeloma. Despite the effectiveness of benzamide derivatives in treating other cancers, there are few studies on their use in multiple myeloma (MM) cell lines. Consequently, there is a significant need to develop new benzamide-derived drugs for MM treatment that have novel targets, high efficacy, and minimal side effects. This study aims to highlight the potential of the small benzamide-derived molecule SY15 as a promising new antitumor agent for MM, which warrants further investigation.



N-(4-(benzothiazol-2-ylmethyl)phenyl)benzamide

Figure 1. Structure of SY-15 molecule.

MATERIALS AND METHODS

Cell culture

In this study, four different MM cell lines (MM1S, U266, H929, and RPMI 8226) and the L929 normal fibroblast cell line were used. All cell lines were cultured in Roswell Park Memorial Institute medium (RPMI-1640, with L-glutamine) (Sigma, USA) supplemented with 10% fetal bovine serum (FBS) (Sigma, USA) and antibiotics (Penicillin 100 U/mL and Streptomycin 100 µg/mL) (Gibco, USA). The cells were maintained under standard conditions in an incubator at 37°C with a gas mixture of 5% CO₂ and 95% air. Myeloma cells were maintained by adding 5 mL of fresh medium every two days until reaching 80% confluency.

MM.1S and RPMI-8226 cell lines are semi-adherent; therefore, cells must be carefully scraped before passaging. L929 cells are adherent cells and were detached by incubating with 2–3 mL of Trypsin-EDTA for 4 minutes. Viable cell counting was performed using the Trypan blue exclusion method with a hemocytometer. When the percentage of viable cells exceeded 85%, the cultures were considered ready for experiments [15].

MTT assay

The cytotoxic effects of the SY15 molecule were evaluated using the MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) assay (Roche, Germany). Cells were seeded into 96-well plates at a density of 5×10^4 cells per well and incubated overnight. Then, different concentrations of the SY15 molecule (1, 5, 10, 15, 25, 50, 75, and 100 µM) were applied to the cells and incubated for 72 hours. At the end of the incubation period, 10 µL of MTT solution (5 mg/mL) was added to each well. After a four-hour incubation, 100 µL of Sodium Dodecyl Sulfate-Hydrochloric acid (SDS-HCl) (0.01 M) solution was added to dissolve the formazan crystals. Optical density was measured at 550 nm with a reference wavelength of 690 nm using a spectrophotometric reader (Biotek, USA). Cell viability was calculated as a percentage relative to the negative control (cells treated with DMSO). Small changes in metabolic activity lead to significant changes in MTT, allowing detection of cellular stress even without direct cell death caused by toxic agents. This method is standardized for adherent or non-adherent cells grown in multi-well plates [16]. The data obtained at the end of 72 hours were analyzed.

Sample size

To detect an effect size of 0.31 (Cohen's *f*) for vitality percentage among cell lines with a significance level of 0.05 and a power of 0.80, a minimum total sample size of 130 (i.e., 26 for each cell line) is required. The sample size calculation was performed using G*Power (version 3.1.9.4).

Statistical analysis

Descriptive statistics for continuous variables are presented as the median (minimum, maximum). The Shapiro-Wilk test

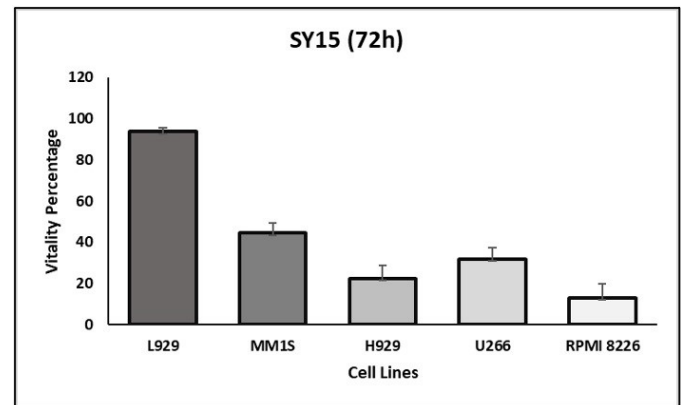


Figure 2. Viability percentages among five cell lines.

was used to assess the normality of the data. To determine significant differences between groups, the Kruskal-Wallis test was employed, followed by the Dunn's post hoc test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant. All analyses were conducted using the Statistical Package for the Social Sciences (SPSS, version 30.0, Chicago, IL) and JASP (version 0.18).

RESULTS

At the end of the 72nd hour, the activity of the SY-15 molecule in multiple myeloma cells was examined. A statistically significant difference was observed among the five cell lines in terms of vitality percentages ($p < 0.001$) (Figure 2). The median vitality percentage of the L929 cell line was 93.6247 (83.9708, 126.4116), that of the MM1S cell line was 44.4110 (27.2785, 105.3916), H929 had a median of 22.4655 (12.4898, 103.4063), U266 had 31.7180 (27.2775, 103.9295), and RPMI 8226 showed the lowest median value of 13.0931 (11.1188, 101.8358). Statistically significant differences between groups were observed for the following comparisons, RPMI 8226 vs U266 ($p = 0.003$), RPMI 8226 vs MM1S ($p < 0.001$), RPMI 8226 vs L929 ($p < 0.001$), RPMI 8226 vs MM1S ($p = 0.002$), RPMI 8226 vs L929 ($p < 0.001$), H929 vs L929 ($p < 0.001$) and U266 vs L929 ($p = 0.005$) (Table 1) (Figure 3).

A significant difference in vitality percentage was observed between the doses in the L929 cell line ($p = 0.144$) (Figure 4a). The median vitality percentage at the 0 µM dose was 102.1857 (83.9708, 113.8433), 1 µM dose was 110.0182 (109.6539, 126.4116), 5 µM dose was 102.7322 (93.4426, 108.0145), 10 µM dose was 99.6357 (92.1675, 100.1821), 15 µM dose was 93.2604 (91.8032, 95.0819), 25 µM dose was 92.1675 (91.4389, 100.7285), 50 µM dose was 92.7140 (88.7067, 93.6247), 75 µM dose was 93.0783 (92.5318, 97.2677) and 100 µM dose was 88.8888 (84.1530, 94.1712) (Table 2).

A significant difference in vitality percentage was observed between the doses in the MM1S cell line ($p = 0.005$) (Figure 4b). The median vitality percentage at the 0 µM dose was 99.8684 (98.6285, 105.3916); at 1 µM, it was 99.1921 (92.9926,

Table 1. Viability percentages among five cell lines.

	L929	MM1S	Cell Lines H929	U266	RPMI 8226	p value
Vitality Percentage	93.6247	44.4110	22.4655	31.7180	13.0931	<0.001 ^α
Median (min-max)	(83.9708-126.4116)	(27.2785-105.3916)	(12.4898-103.4063)	(27.2775-103.9295)	(11.1188-101.8358)	

min: minimum max:maximum, ^α Kruskal-Wallis Test.**Table 2.** Percentage of viability among all doses in all cell lines.

Doses	L929	MM1S	Cell Lines H929	U266	RPMI 8226
0 μM	102.1857 (83.9708-113.8433)	99.8684 (98.6285-105.3916)	103.0819 (102.3519-103.4063)	99.4889 (99.3832-99.9118)	101.5240 (96.6401-101.8358)
1 μM	110.0182 (109.6539-126.4116)	99.1921 (92.9926-100.7702)	87.3479 (81.3463-90.9975)	97.1623 (83.3127-103.9295)	88.2230 (84.6899-93.4187)
5 μM	102.7322 (93.4426-108.0145)	58.9517 (56.4719-71.6888)	41.0381 (36.9018-44.4444)	59.3127 (55.8237-61.6387)	19.1201 (18.9123-21.1984)
10 μM	99.6357 (92.1675-100.1821)	46.1018 (44.4110-63.2350)	28.7915 (24.4120-29.4403)	34.0440 (31.7180-34.8898)	13.0931 (11.1188-13.9244)
15 μM	93.2604 (91.8032-95.0819)	44.0728 (35.6190-53.9921)	22.4655 (18.8158-25.7907)	32.6696 (30.6607-32.8810)	12.4696 (12.3657-12.9892)
25 μM	92.1675 (91.4389-100.7285)	43.9601 (33.2519-44.8619)	17.7615 (17.2749-19.0592)	29.4977 (27.2775-29.6035)	12.3657 (12.1579-12.3657)
50 μM	92.7140 (88.7067-93.6247)	38.0988 (33.3646-38.6624)	17.2749 (13.2197-21.5733)	28.1233 (27.9111-28.1233)	12.4696 (12.0540-12.5736)
75 μM	93.0783 (92.5318-97.2677)	31.8993 (27.2778-39.0000)	17.7615 (14.7607-18.4914)	28.4405 (28.2290-28.9691)	12.0540 (11.8462-12.2618)
100 μM	88.8888 (84.1530-94.1712)	33.3646 (29.8703-35.84444)	19.7891 (14.4898-20.5190)	27.2775 (27.2775-27.9118)	14.0284 (13.8205-15.0675)
p value	0.144 ^α	0.005 ^α	0.004 ^α	0.002 ^α	0.004 ^α

min: minimum max:maximum, ^α Kruskal-Wallis Test. Descriptive statistics are presented as median (min-max).

100.7702); at 5 μM, it was 58.9517 (56.4719, 71.6888); at 10 μM, it was 46.1018 (44.4110, 63.2350); at 15 μM, it was 44.0728 (35.6190, 53.9921); at 25 μM, it was 43.9601 (33.2519, 44.8619); at 50 μM, it was 38.0988 (33.3646, 38.6624); at 75 μM, it was 31.8993 (27.2778, 39.0000); and at 100 μM, it was 33.3646 (29.8703, 35.84444). The lowest vitality percentage was observed at the 75 μM dose. Statistically significant differences between groups were found in the following comparisons: 0 μM vs 25 μM (p=0.024), 0 μM vs 50 μM (p=0.009), 0 μM vs 75 μM (p=0.002), 0 μM vs 100 μM (p=0.002), 1 μM vs 25 μM (p=0.035), 1 μM vs 50 μM (p=0.015), 1 μM vs 75 μM (p=0.003), 1 μM vs 100 μM (p=0.004), 5 μM vs 75 μM (p=0.027), and 5 μM vs 100 μM (p=0.029) (Table 2).

A significant difference in vitality percentage was observed between the doses in the H929 cell line (p=0.004) (Figure 4c). The median vitality percentage at 0 μM was 103.0819 (102.3519, 103.4063), at 1 μM was 87.3479 (81.3463, 90.9975), at 5 μM was 41.0381 (36.9018, 44.4444), at 10 μM was 28.7915 (24.4120, 29.4403), at 15 μM was 22.4655 (18.8158, 25.7907), at 25 μM was 17.7615 (17.2749, 19.0592), at 50 μM was 17.2749 (13.2197, 21.5733), at 75

μM was 17.7615 (14.7607, 18.4914), and at 100 μM was 19.7891 (14.4898, 20.5190). The lowest vitality percentage was observed at the 50 μM dose. Statistically significant differences between groups were found in the following comparisons: 0 μM vs 25 μM (p=0.008), 0 μM vs 50 μM (p=0.006), 0 μM vs 75 μM (p=0.005), 0 μM vs 100 μM (p=0.012), 1 μM vs 25 μM (p=0.01), 1 μM vs 50 μM (p=0.008), 1 μM vs 75 μM (p=0.006), 1 μM vs 100 μM (p=0.016), 5 μM vs 25 μM (p=0.037), 5 μM vs 50 μM (p=0.031), and 5 μM vs 75 μM (p=0.023) (Table 2).

A significant difference in vitality percentage was observed between the doses in the U266 cell line (p=0.002) (Figure 4d). The median vitality percentage at the 0 μM dose was 99.4889 (99.3832, 99.9118). At 1 μM, it was 97.1623 (83.3127, 103.9295), at 5 μM, 59.3127 (55.8237, 61.6387), at 10 μM, 34.0440 (31.7180, 34.8898), at 15 μM, 32.6696 (30.6607, 32.8810), at 25 μM, 29.4977 (27.2775, 29.6035), at 50 μM, 28.1233 (27.9111, 28.1233), at 75 μM, 28.4405 (28.2290, 28.9691), and at 100 μM, 27.2775 (27.2775, 27.9118). The lowest vitality percentage was observed at 100 μM. Statistically significant differences between groups included 0 μM vs 25 μM (p=0.01), 0 μM vs 50 μM (p=0.003), 0 μM vs

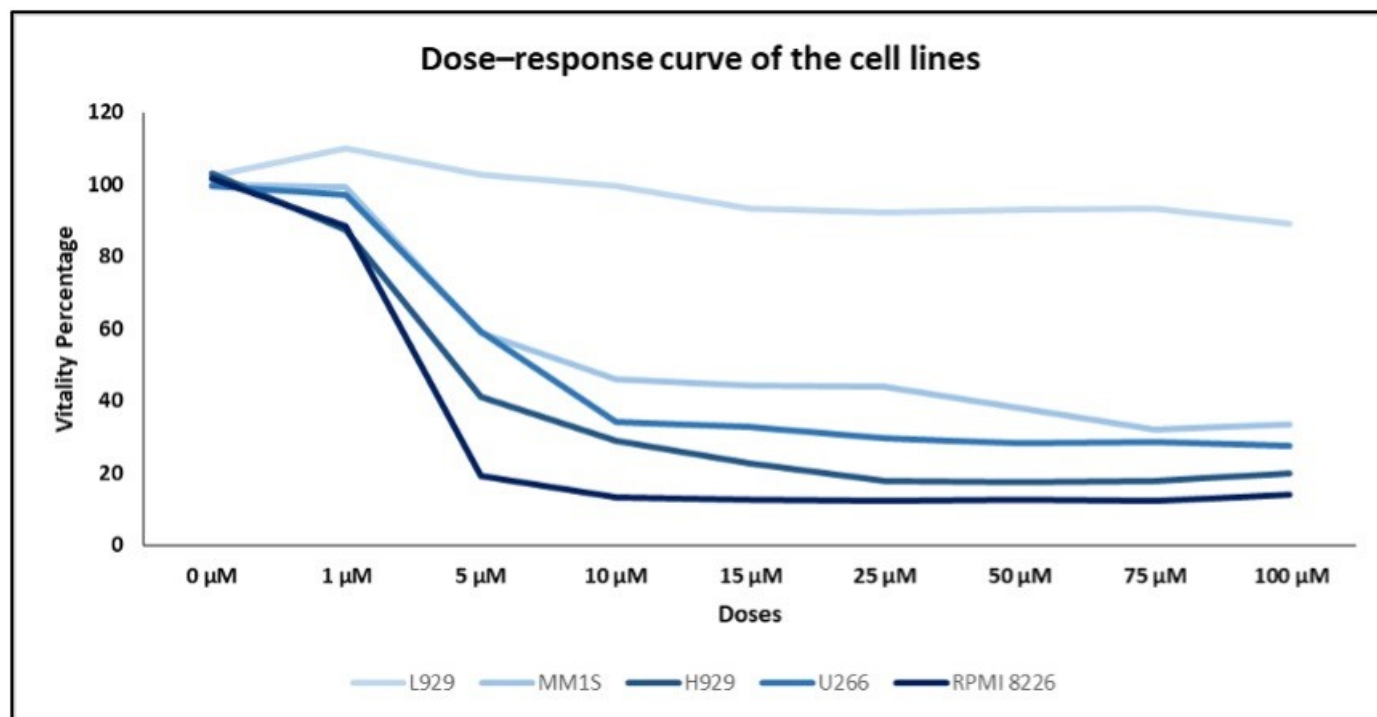


Figure 3. Dose-response curves in all cell lines at all doses.

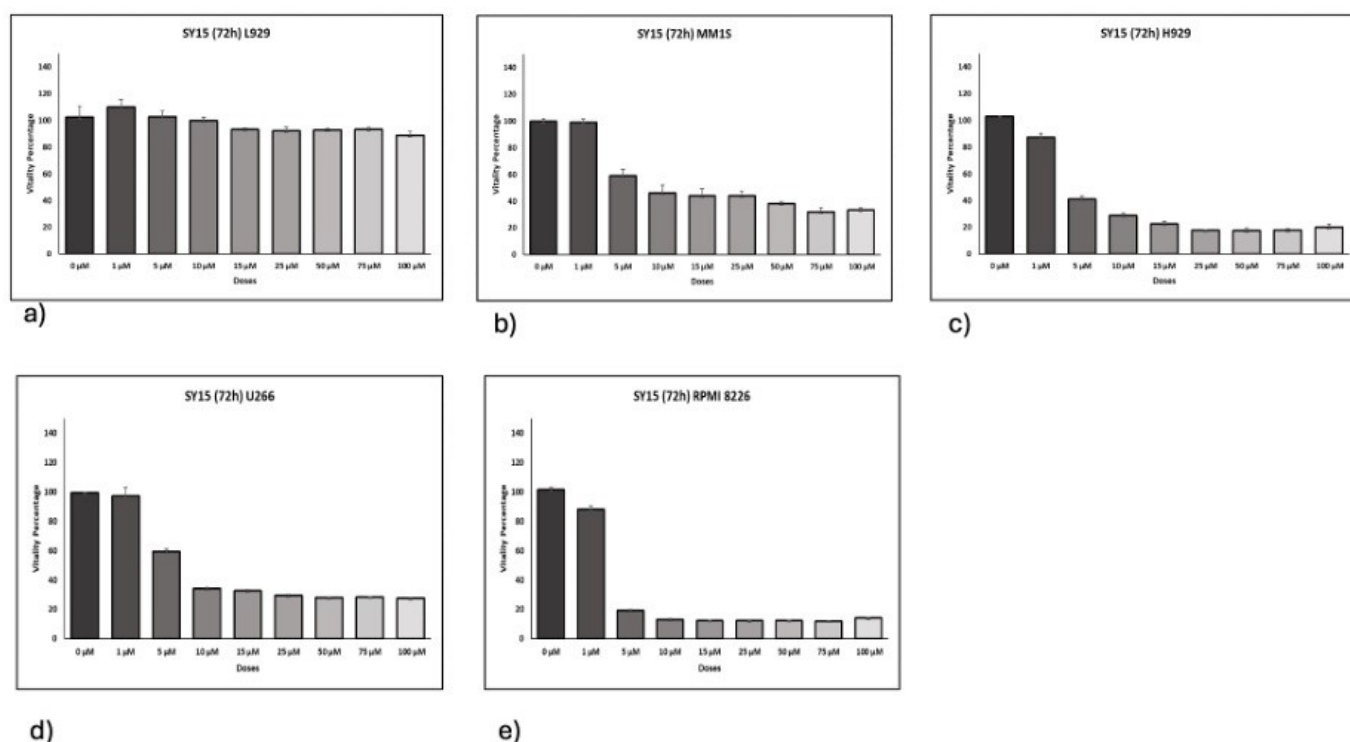


Figure 4. a) Percentage of viability between doses in L929 cell line; b) Percentage of viability between doses in MM1S cell line; c) Percentage of viability between doses in H929 cell line; d) Percentage of viability between doses in U266 cell line; e) Percentage of viability between doses in RPMI8226 cell line.

75 μM ($p=0.013$), 0 μM vs 100 μM ($p<0.001$), 1 μM vs 25 μM ($p=0.016$), 1 μM vs 50 μM ($p=0.005$), 1 μM vs 75 μM ($p=0.021$), 1 μM vs 100 μM ($p=0.001$), 5 μM vs 50 μM ($p=0.029$), 5 μM vs 100 μM ($p=0.008$), and 10 μM vs 100

μM ($p=0.037$) (Table 2).

A significant difference in vitality percentage was observed between the doses in the RPMI 8226 cell line ($p=0.004$; Figure 4e). The median vitality percentage at the 0 μM dose

was 101.5240 (96.6401, 101.8358), at 1 μ M was 88.2230 (84.6899, 93.4187), at 5 μ M was 19.1201 (18.9123, 21.1984), at 10 μ M was 13.0931 (11.1188, 13.9244), at 15 μ M was 12.4696 (12.3657, 12.9892), at 25 μ M was 12.3657 (12.1579, 12.3657), at 50 μ M was 12.4696 (12.0540, 12.5736), at 75 μ M was 12.0540 (11.8462, 12.2618), and at 100 μ M was 14.0284 (13.8205, 15.0675). The lowest vitality percentage was observed at the 75 μ M dose. Statistically significant differences between groups were noted for the following comparisons: 0 μ M vs 10 μ M ($p=0.016$), 0 μ M vs 15 μ M ($p=0.017$), 0 μ M vs 25 μ M ($p=0.003$), 0 μ M vs 50 μ M ($p=0.007$), 0 μ M vs 75 μ M ($p<0.001$), 1 μ M vs 25 μ M ($p=0.013$), 1 μ M vs 50 μ M ($p=0.027$), 1 μ M vs 75 μ M ($p=0.003$), 5 μ M vs 25 μ M ($p=0.045$), and 5 μ M vs 75 μ M ($p=0.013$) (Table 2).

■ DISCUSSION

The treatment of multiple myeloma (MM) involves using a combination of drugs, each producing different responses. These include corticosteroids, alkylating agents, anthracyclines, proteasome inhibitors (PIs), immunomodulatory drugs (IMiDs), histone deacetylase inhibitors (HDAC inhibitors), monoclonal antibodies (mAbs), and nuclear export inhibitors [17]. For a long time, especially, the alkylating agent melphalan and the corticosteroid prednisone were the main cytotoxic drugs used in treatment [18]. Later, high-dose melphalan chemotherapy followed by autologous stem cell transplantation began to be used, and this method was shown to extend survival in younger patients compared to conventional chemotherapy [19]. Various combinations of melphalan and prednisone became the most common treatment for elderly patients. Subsequently, thalidomide, bortezomib, and lenalidomide were added to treatment protocols for these patients [20]. One of the most important drugs in MM treatment, bortezomib, was the first proteasome inhibitor deemed suitable for treating relapsed and refractory MM patients [21, 22]. The introduction of this PI has become the most effective treatment against multiple myeloma by preventing pro-apoptotic protein degradation and promoting tumor cell death. Using these drugs in different formats has improved the average survival of patients with multiple myeloma [23]. However, adverse events have been noted in MM patients who develop resistance to both first-generation IMiDs and proteasome inhibitors [24].

Among the recently discovered anticancer drugs, various benzimidazole derivatives have received particular attention. The benzamide derivative MS-247, synthesized by Yamori and colleagues, showed antitumor activity in 39 cancer cell lines and many organ tissue cells. It binds to AT-rich regions in the minor groove of DNA, inhibits DNA synthesis, creates inter-strand crosslinks (ICLs), blocks the cell cycle in the G2/M phase, and induces apoptosis [25]. In a recent study, a new benzamide derivative called VKNG-2 was shown to enhance the effectiveness of chemotherapeutic drugs in colon cancer cell lines by inhibiting the ABCG2 transporter [26]. In an-

other study, a novel benzamide derivative containing benzamidophenyl and phenylacetamidophenyl scaffolds, known as 13f, exhibited strong anticancer activity and a potent PARP-1 inhibitory effect against human colorectal cancer HCT116 and DLD-1 cells. This compound effectively inhibited colony formation and migration in HCT116 cells [27]. In a separate study, N-(9H-purin-6-yl) benzamide derivatives were reported to induce apoptosis and reduce cell proliferation in cancer cell lines, showing cytotoxic and antitumor activity within the range of 3–39 μ M [28]. In another recent study, the effect of an imidazole derivative on A549 lung cancer cells was investigated. The compound was found to exhibit anticancer activity by inhibiting cell proliferation and accelerating apoptosis [29]. A new series of 2-amino-1,4-naphthoquinone-benzamide derivatives, labeled 5a–n, was tested across three different cancer cell lines. Results showed that these compounds were most effective in the MDA-MB-231 cell line, less effective in the SUIT-2 cell line, and more effective than the positive control cisplatin in the HT-29 cell line [30]. Following the combined treatment of the benzamide derivative XT5 and imatinib in K562 cell lines, an increase in cytotoxicity was observed, along with Annexin V binding and caspase 3/7 activation. Expression levels of pro-apoptotic genes also increased in K562R and K562S cell lines treated with XT5. While XT2B did not form hydrogen bonds, XT5 showed hydrogen bond interactions with the basic amino acids of the BCR-ABL kinase receptor [31].

Recently synthesized (E)-N-phenyl-4-(pyridine-acylhydrazone) benzamide derivatives were tested for their antiproliferative activity against U266 and RPMI 8226 cell lines using the MTT assay. Compound 8b showed excellent antiproliferative activity against RPMI 8226 cells, had lower toxicity than imatinib, significantly halted the cell cycle in the G0/G1 phase, and induced cell death in RPMI 8226 cells by increasing mitochondrial ROS release, thus producing an antitumor effect [32].

■ CONCLUSION

Despite the potential of benzamide derivatives to serve as highly effective antitumor drug candidates across various cancer types, few studies have been conducted on MM cell lines. This research contributes to the development of new, potent anticancer agents with high selectivity and low toxicity. The SY-15 molecule exhibits several distinct features that set it apart from other benzamide derivatives and traditional HDAC inhibitors. Notably, it demonstrates significant cytotoxic activity in multiple MM cell lines, including those sensitive to bortezomib. Structurally, SY-15 incorporates a benzothiazole moiety linked to its benzamide core, which may enhance binding affinity through hydrophobic interactions, pi-pi stacking, and hydrogen bonding. This structural characteristic could help explain its selectivity and potency. Additionally, while most classical benzamide derivatives mainly target HDAC enzymes, SY-15 may exert antitumor effects not

only by inhibiting HDACs but also by modulating alternative apoptotic pathways specific to multiple myeloma cells. Its low cytotoxicity in normal fibroblast cells indicates that SY-15 has a more favorable therapeutic index compared to other existing HDAC inhibitors. This study lays a solid foundation for further evaluation and target identification of SY-15. Future research will focus on testing the efficacy of the benzamide-derived compound in bortezomib-resistant MM cell lines. This will be a highly exciting development, as it will be the first study to target benzamide derivatives in resistant MM cell lines. Detailed efficacy studies of this drug alone or in combination with bortezomib in resistant cell lines could yield better results and potentially offer a new treatment option as an effective anticancer drug for MM therapy, especially in resistant patients.

Ethics Committee Approval: Since this study was conducted on cell lines (in vitro), ethical committee approval was not required.

Informed Consent: In this study, no patient or volunteer was involved, so an informed consent form was not required.

Peer-review: Externally peer-reviewed.

Conflict of Interest: All authors have disclosed no conflicts of interest.

Author Contributions: N.A: Conception, Design, Supervision, Literature Review, Data Collection and/or Processing, Analysis and/or Interpretation; K.G: Writing; N.F: Materials; E.D: Statistical Analysis; S.O: Molecular Synthesis; A.S: Interpretation and Critical Review.

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■ REFERENCES

- Chesi M, Bergsagel PL. Advances in the pathogenesis and diagnosis of multiple myeloma. *Int J Lab Hematol*. 2015;37 Suppl 1:108-14. doi: [10.1111/ijlh.12360](https://doi.org/10.1111/ijlh.12360).
- Rajkumar SV, Dimopoulos MA, Palumbo A, Blade J, Merlini G, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. *Lancet Oncol*. 2014;15(12):e538-48. doi: [10.1016/S1470-2045\(14\)70442-5](https://doi.org/10.1016/S1470-2045(14)70442-5).
- <https://www.cancer.org/cancer/types/multiple-myeloma.html>.
- Rajkumar SV. Multiple myeloma: 2020 update on diagnosis, risk-stratification and management. *Am J Hematol*. 2020;95(5):548-567. doi: [10.1002/ajh.25791](https://doi.org/10.1002/ajh.25791).
- Greipp PR, Miguel JS, Durie BGM. International staging system for multiple myeloma. *J Clin Oncol*. 2005;23(15):3412–3420. doi: [10.1200/JCO.2005.04.242](https://doi.org/10.1200/JCO.2005.04.242).
- Ross JA, Avet-Loiseau H, Li X, Thiebaut-Millot R, Hader C. Genomic landscape of t(11;14) in multiple myeloma. *Blood*. 2022;140(Supplement 1):10092–10093. doi: [10.1182/blood-2022-167167](https://doi.org/10.1182/blood-2022-167167).
- Wiedmeier-Nutor JE, Bergsagel PL. Review of Multiple Myeloma Genetics including Effects on Prognosis, Response to Treatment, and Diagnostic Workup. *Life (Basel)*. 2022 May 30;12(6):812. doi: [10.3390/life12060812](https://doi.org/10.3390/life12060812).
- Bal S, Kumar SK, Fonseca R. Multiple myeloma with t(11;14): unique biology and evolving landscape. *Am J Cancer Res*. 2022;12(7):2950–2965. PMID: [35968339](https://pubmed.ncbi.nlm.nih.gov/35968339/).
- Tsubaki M, Takeda T, Ogawa N, Sakamoto K, Shimaoka H, et al. Overexpression of survivin via activation of ERK1/2, Akt, and NF- κ B plays a central role in vincristine resistance in multiple myeloma cells. *Leuk Res*. 2015;39(4):445-52. doi: [10.1016/j.leukres.2015.01.016](https://doi.org/10.1016/j.leukres.2015.01.016).
- Xu X, Liu J, Shen C, Ding L, Zhong F, et al. The role of ubiquitin-specific protease 14 (USP14) in cell adhesion-mediated drug resistance (CAM-DR) of multiple myeloma cells. *Eur J Haematol*. 2017;98(1):4-12. doi: [10.1111/ejh.12729](https://doi.org/10.1111/ejh.12729).
- Fernandes GFDS, Fernandes BC, Valente V, Dos Santos JL. Recent advances in the discovery of small molecules targeting glioblastoma. *Eur J Med Chem*. 2019;164:8-26. doi: [10.1016/j.ejmech.2018.12.033](https://doi.org/10.1016/j.ejmech.2018.12.033).
- Günkara ÖT. Antikanser Aktivite Gösterebilecek Yeni Heterohalkalı Bileşiklerin Sentezlenmesi Ve Karakterizasyonu. *JEPS*. 2019;31(1):83-9. doi: [10.7240/jeps.494603](https://doi.org/10.7240/jeps.494603).
- Movafagh S, Munson A. Histone deacetylase inhibitors in cancer prevention and therapy. *Academic Press*. 2019:75-105. doi: [10.1016/B978-0-12-812494-9.00004-4](https://doi.org/10.1016/B978-0-12-812494-9.00004-4).
- Yilmaz S, Yalcin I, Kaynak-Onurdag F, Ozgen S, Yildiz I, et al. Synthesis and in vitro antimicrobial activity of novel 2-(4-(substituted-carboxamido) benzyl/phenyl) benzothiazoles. *Croatica Chemica Acta*. 2013;86(2):23-231. doi: [10.5562/cca2064](https://doi.org/10.5562/cca2064).
- Fairfield H, Condruti R, Farrell M, Di Iorio R, Gartner CA, et al. Development and characterization of three cell culture systems to investigate the relationship between primary bone marrow adipocytes and myeloma cells. *Front Oncol*. 2023;12:912834. doi: [10.3389/fonc.2022.912834](https://doi.org/10.3389/fonc.2022.912834).
- Kumar P, Nagarajan A, Uchil PD. Analysis of Cell Viability by the MTT Assay. *Cold Spring Harb Protoc*. 2018;2018(6). doi: [10.1101/pdb.prot095505](https://doi.org/10.1101/pdb.prot095505).
- Rajkumar SV. Multiple myeloma: Every year a new standard? *Hematol Oncol*. 2019;37(Suppl 1):62-65. doi: [10.1002/hon.2586](https://doi.org/10.1002/hon.2586).
- Kyle RA, Rajkumar SV. Treatment of multiple myeloma: a comprehensive review. *Clin Lymphoma Myeloma*. 2009;9(4):278-88. doi: [10.3816/CLM.2009.n.056](https://doi.org/10.3816/CLM.2009.n.056).
- Harousseau JL, Moreau P. Autologous hematopoietic stem-cell transplantation for multiple myeloma. *N Engl J Med*. 2009;360(25):2645-54. doi: [10.1056/NEJMct0805626](https://doi.org/10.1056/NEJMct0805626).
- Mina R, Brighen S, Wildes TM, Zweegman S, Rosko AE. Approach to the Older Adult With Multiple Myeloma. *Am Soc Clin Oncol Educ Book*. 2019;39:500-518. doi: [10.1200/EDBK_239067](https://doi.org/10.1200/EDBK_239067).
- Richardson PG, Sonneveld P, Schuster MW, Irwin D, Stadtmauer EA, et al. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. *N Engl J Med*. 2005;352(24):2487-98. doi: [10.1056/NEJMoa043445](https://doi.org/10.1056/NEJMoa043445).
- Chen D, Frezza M, Schmitt S, Kanwar J, Dou QP. Bortezomib as the first proteasome inhibitor anticancer drug: current status and future perspectives. *Curr Cancer Drug Targets*. 2011;11(3):239-53. doi: [10.2174/156800911794519752](https://doi.org/10.2174/156800911794519752).
- Rajkumar SV. Treatment of multiple myeloma. *Nat Rev Clin Oncol*. 2011;8(8):479-91. doi: [10.1038/nrclinonc.2011.63](https://doi.org/10.1038/nrclinonc.2011.63).
- Kumar SK, Lee JH, Lahuerta JJ, Morgan G, Richardson PG, et al. International Myeloma Working Group. Risk of progression and survival in multiple myeloma relapsing after therapy with IMiDs and bortezomib: a multicenter international myeloma working group study. *Leukemia*. 2012;26(1):149-57. doi: [10.1038/leu.2011.196](https://doi.org/10.1038/leu.2011.196).
- Matsuba Y, Edatsugi H, Mita I, Matsunaga A, Nakanishi O. A novel synthetic DNA minor groove binder, MS-247: antitumor activity and cytotoxic mechanism. *Cancer Chemother Pharmacol*. 2000;46(1):1-9. doi: [10.1007/s002800000120](https://doi.org/10.1007/s002800000120).
- Narayanan S, Gujarati NA, Wang JQ, Wu ZX, Koya J, et al. The Novel Benzamide Derivative, VKNG-2, Restores the Efficacy of Chemotherapeutic Drugs in Colon Cancer Cell Lines by Inhibiting the ABCG2 Transporter. *Int J Mol Sci*. 2021;22(5):2463. doi: [10.3390/ijms22052463](https://doi.org/10.3390/ijms22052463).

27. Lu G, Nie W, Xin M, Meng Y, Jiang J, et al. Discovery of novel benzamide derivatives bearing benzamidophenyl and phenylacetamidophenyl scaffolds as potential antitumor agents via targeting PARP-1. *Eur J Med Chem.* 2023;251:115243. doi: [10.1016/j.ejmech.2023.115243](https://doi.org/10.1016/j.ejmech.2023.115243).
28. Cros-Perrial E, Saulnier S, Raza MZ, Charmelot R, Egron D, et al. Cytotoxic and Antitumoral Activity of N-(9H-purin-6-yl) Benzamide Derivatives and Related Water-soluble Prodrugs. *Curr Mol Pharmacol.* 2022;15(6):883-894. doi: [10.2174/1874467214666211014164406](https://doi.org/10.2174/1874467214666211014164406).
29. Bhat SA, Pajaniradje S, Bhunia S, Subramanian S, Chandramohan S, et al. A study on the anticancer activity of imidazolyl benzamide derivative-IMUEB on a 549 lung cancer cell line. *J Cancer Res Ther.* 2023;19(5):1288-1296. doi: [10.4103/jcrt.jcrt_1788_21](https://doi.org/10.4103/jcrt.jcrt_1788_21).
30. Sayahi MH, Hassani B, Mohammadi-Khanaposhtani M, Dastyafteh N, Gohari MR, et al. Design, synthesis, and cytotoxic activity of 2-amino-1,4-naphthoquinone-benzamide derivatives as apoptosis inducers. *Sci Rep.* 2024;14(1):27302. doi: [10.1038/s41598-024-78468-2](https://doi.org/10.1038/s41598-024-78468-2).
31. Ozkan T, Hekmatshoar Y, Ertan-Bolelli T, Hidayat AN, Beksac M, et al. Determination of the Apoptotic Effect and Molecular Docking of Benzamide Derivative XT5 in K562 Cells. *Anticancer Agents Med Chem.* 2018;18(11):1521-1530. doi: [10.2174/1871520618666171229222534](https://doi.org/10.2174/1871520618666171229222534).
32. Li XY, Li S, Lu GQ, Wang DP, Liu KL, et al. Design, synthesis and biological evaluation of novel (E)-N-phenyl-4-(pyridine-acylhydrazone) benzamide derivatives as potential antitumor agents for the treatment of multiple myeloma (MM). *Bioorg Chem.* 2020;103:104189. doi: [10.1016/j.bioorg.2020.104189](https://doi.org/10.1016/j.bioorg.2020.104189).



The relationship between lung involvement in rheumatoid arthritis and sarcopenia

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■ MAIN POINTS

- Lung involvement of Rheumatoid Arthritis affects the quality of muscle.
- Computed tomography density is a valuable method for determining muscle quality.
- The muscle density/ aortic density ratio allows assessment of muscle quality without being influenced by acquisition parameters.

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■ ABSTRACT

Aim: This study investigated the effect of lung involvement in Rheumatoid Arthritis (RA) on sarcopenia.

Materials and Methods: A retrospective analysis was conducted on patients with RA diagnosed between January 2020 and January 2024. Patients with non-contrast thoracic CT scans available in the hospital database were included. A control group consisting of individuals without any inflammatory diseases was also selected. Muscle area (MA) and muscle-to-aorta density (M/A density) ratio were compared between the RA and control groups, as well as between RA patients with and without pulmonary involvement.

Results: A total of 187 individuals (156 women, 31 men) were included in the study, comprising 84 patients in the RA group and 103 in the control group. The M/A density ratio was significantly lower in the RA group ($p < 0.001$). Among RA patients, 30 (36%) had pulmonary involvement. The M/A density ratio was significantly lower in RA patients with lung involvement compared to those without ($p = 0.016$). However, the muscle area showed no significant difference among the groups ($p = 0.683$).

Conclusion: This study found that RA lung involvement may be associated with increased muscle adiposity. Prospective studies with large populations are needed to confirm this association.

Keywords: Rheumatoid Arthritis (RA), Lung involvement, Sarcopenia, Muscle density, Computed Tomography (CT)

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■ INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects the joints. The prevalence of rheumatoid arthritis varies worldwide, ranging from 0.25% to 1%. Women are affected more [1]. Proinflammatory cytokines such as tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), and interleukin-1 (IL-1) play a key role in the pathogenesis of RA [2]. In approximately half of the patients with RA, concomitant extraarticular findings can be present. The respiratory system is involved in 60-80% of RA patients, which is the most common extraarticular involvement [3].

Sarcopenia is a disease that reduces muscle strength, mass, and function. Usually, it occurs later in life [2]. A revised criteria system, developed by the European Working Group on Sarcopenia in Older People 2 (EWGSOP2) in 2018, updated the

definition of sarcopenia. EWGSOP2 defined two types of sarcopenia: primary sarcopenia associated with aging in adults over 40 years old, and secondary sarcopenia related to chronic inflammation, inactivity, or malnutrition. EWGSOP2 has also identified Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) as the most suitable diagnostic imaging methods for measuring muscle area [4]. In primary sarcopenia, various factors, including physical inactivity, inflammation, and hormonal changes, contribute. These causes are also effective in rheumatoid sarcopenia. Proinflammatory cytokines in RA may accelerate muscle loss, leading to the progression of rheumatoid sarcopenia. Additionally, decreased physical activity further increases this risk [5].

In the RA population, the number of studies using CT to evaluate the muscle area or density of the RA is very scarce.

Although the effect of RA on muscle tissue has been examined in the literature, the relationship between lung involvement in RA and its relation with muscle density has not been previously evaluated. This study is one of the first to examine how muscle density is affected in patients with RA lung involvement. For these reasons, this study aimed to investigate the relationship between sarcopenia and pulmonary involvement in RA, which is common in the Turkish population, by measuring the paraspinal muscle area on CT.

■ MATERIALS AND METHODS

Study design

This retrospective, observational case-control study was conducted according to the STROBE guidelines.

Sampling method

A non-probability purposive sampling method was employed to include RA patients with thoracic CT scans available between January 2020 and January 2024. After obtaining ethics committee approval, patients diagnosed with RA in our hospital were retrospectively screened. Among these patients, those with non-contrast Thorax CT were included in the study. Age- and sex-matched controls without inflammatory diseases were selected using simple random sampling from the hospital database. The control group was selected from pre-diagnoses without widespread systemic effects such as trauma or nodule follow-up. In both groups, a diagnosis of cancer, a history of chronic diseases known to affect muscle health (e.g., chronic kidney disease, liver disease, malabsorption syndromes), or pregnancy was excluded. In addition, those whose CT images had artifacts that were enough to affect the evaluation were also excluded from the study. CRP, RF, and anti-CCP values of patients with RA were also examined. Thorax CTs were evaluated for rheumatoid arthritis lung involvement in the group diagnosed with RA. Lung involvement patterns were determined as usual interstitial pneumonia (UIP), non-specific interstitial pneumonia (NSIP), bronchiolitis obliterans (BO), rheumatoid nodule (RN), isolated bronchiectasis, and lymphoid interstitial pneumonia (LIP) [6].

Sample size

Due to the retrospective design, sample size was determined by the availability of suitable patients and controls within the hospital records. Sample size calculation was performed.

Measurement methods

Paraspinal muscle area (MA) was manually measured on axial thoracic CT images at the level of the 12th thoracic vertebra using the Sectra PACS (IDS7, Sweden) software. A region of interest (ROI) was carefully drawn to encompass the outer margin of the paraspinal muscles bilaterally. The aortic density was measured by placing an ROI over the aorta at the same

level, using the widest circular area that fully enclosed the lumen without including surrounding tissues. In addition, the degree of muscle adiposity was determined by the ratio of the mean density of the muscle to the aortic density (M/A density) (Figure 1). This ratio eliminated density differences that may arise from shooting parameters. MA and M/A density ratio values were compared between the patient and control groups. In addition, those with and without parenchymal lung involvement in the RA group were compared. All measurements were performed with Sectra Workstation software (PACS, Sectra Workstation IDS7, Sweden). Radiological measurements were independently performed by two experienced radiologists who were blinded to the participants' clinical status and group assignment.



Figure 1. Measurement of muscle area, muscle density, and aortic density.

Since the patient's BMI was unknown, the area of subcutaneous adipose tissue was measured instead (Figure 2). Subcutaneous adipose tissue was evaluated by automatic measurement on the Aquarius iNtuition Viewer (V4.4.13, Terarecon) workstation.

The CT scan was performed using a 128-detector scanner (GE Revolution EVO, Milwaukee, USA). Slices were taken from the lung apices to the bottom. The scan parameters are 0.625mm slice, 100 kV, auto mA (min 80 - max 250), large FOV, 40mm coverage, rotation time 0,6 s, pitch 1,375. The slice thickness was 2.5 mm.

Statistical analysis

Data were analyzed using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY, USA). The normality of data distribution was evaluated using Kolmogorov-Smirnov and Shapiro-Wilk tests. Normally distributed continuous variables were expressed as mean $\pm$ standard deviation; non-normally distributed variables were presented as median (min–max). Categorical variables were reported as

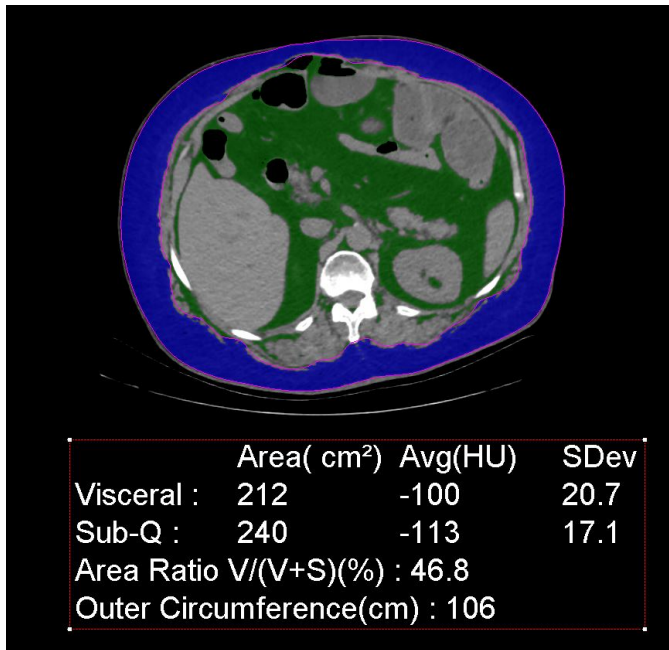


Figure 2. Automatic measurement of subcutaneous fat tissue via workstation.

frequency and percentage. The intraclass correlation coefficient (ICC) was used to assess the interobserver agreement of the MA and M/A density ratio.

For hypothesis testing, the Student's t-test was performed for normally distributed variables, and the Mann-Whitney U test was used for non-normally distributed variables. Spearman correlation analysis was used to assess relationships between continuous variables. Multiple linear regression was used to model the relationship between lung involvement status (dummy-coded) and each muscle outcome, with the control group as the reference category. A p-value < 0.05 was considered statistically significant.

■ RESULTS

This study included 187 people; 156 (83%) were female and 31 (17%) were male. The patient group consisted of 84 individuals, comprising 71 women (85%) and 13 males (15%). The control group consisted of 103 people, including 85 women (83%) and 18 males (17%). There was no significant difference between the two groups in the gender comparison ($p = 0.867$). The interobserver ICC values were 0.970 (95% CI, 0.93–0.98) and 0.991 (95% CI, 0.97–0.99) for MA and the M/A density ratio, respectively.

Comparison of data between groups

CRP values were available in 84 in the RA group and 73 in the control group. Age, CRP, MA, and M/A density ratio data did not follow normal distribution in both groups. Therefore, they were compared with non-parametric tests, and the results are shown in Table 1. Although the median muscle area was higher in the control group and the median CRP in the RA group, no statistical difference was observed

($p=0.180$, $p=0.851$, respectively). M/A density ratio value was significantly lower in the RA group ($p<0.001$) (Figure 3). Subcutaneous fat tissue was normally distributed in both groups. There was no significant difference between the normal and RA groups ($p = 0.088$, as determined by a student t-test).

Table 1. Comparison of the MA and M/A density ratio between the RA group and the control group.

	RA Median (min-max)	Control Median (min-max)	p
Age (year)	60 (26-78)	60 (26-78)	0.952
CRP (mg/dL)	5 (1-215)	5 (1-215)	0.851
MA (mm²)	3260.5 (1090-5347)	3260.5 (1090-5347)	0.180
M/A density ratio	0.52 (-1.60-1.60)	0.52 (-1.60-1.60)	<0.001

p was obtained from the Mann-Whitney U test.

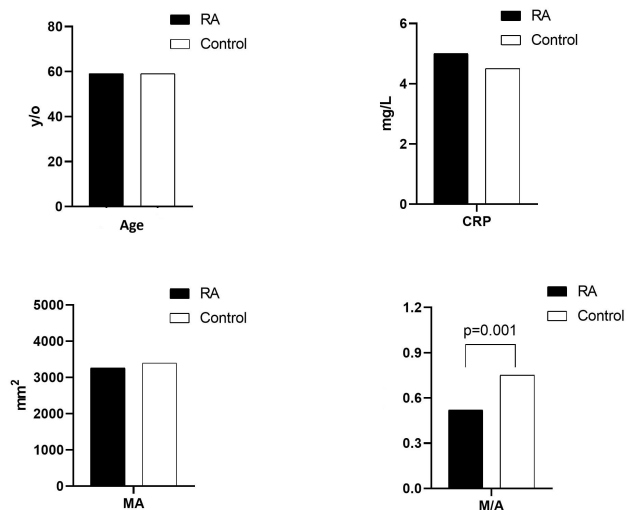


Figure 3. Graphical comparison of RA and control group.

In the RA group, 82 people had Anti-CCP values and median=16 (min=0.5, max=500), 82 had RF values and median=20 (min=1, max=240). 30 (36%) people had lung involvement, and 54 (64%) had no. The involvement pattern was 3 UIP, 9 NSIP, 9 BO, 2 RN, 6 isolated bronchiectasis, and 1 LIP. Isolated emphysema was observed in 2 individuals and was not considered as lung involvement. The patient group was divided into two groups according to lung involvement and compared. RF, CRP, anti-CCP, M/A density, and age were not normally distributed in the patient group. The muscle area and subcutaneous fat tissue followed a normal distribution. There was no significant difference between the groups in terms of gender comparison ($p = 1.000$). There was no significant difference between the patient subgroups when comparing MA and subcutaneous fat tissue using the Student's t-test ($p = 0.683$, $p = 0.779$, respectively). The parameters of the patient sub-group comparison that do not follow a normal distribution are shown in Table 2. In the comparison between those with and without lung involvement,

the M/A density ratio was found to be significantly lower in those with lung involvement ($p < 0.05$) (Figure 4).

Table 2. Comparison of non-normally distributed data of those with and without lung involvement.

	Lung involvement		p
	Yes Median (min-max)	No Median (min-max)	
Age (year)	64 (26-78)	59 (26-74)	0.064
CRP (mg/dL)	4.5 (1-215)	5 (1-116)	0.683
RF (IU/mL)	25 (6-240)	20 (1-159)	0.125
Anti-CCP (EU/ml)	70 (0.5-500)	8.5 (0.5-500)	0.123
M/A density ratio	0.337 (-1.60-1.11)	0.551 (-0.84-1.60)	0.016

p was obtained from the Mann-Whitney U test.

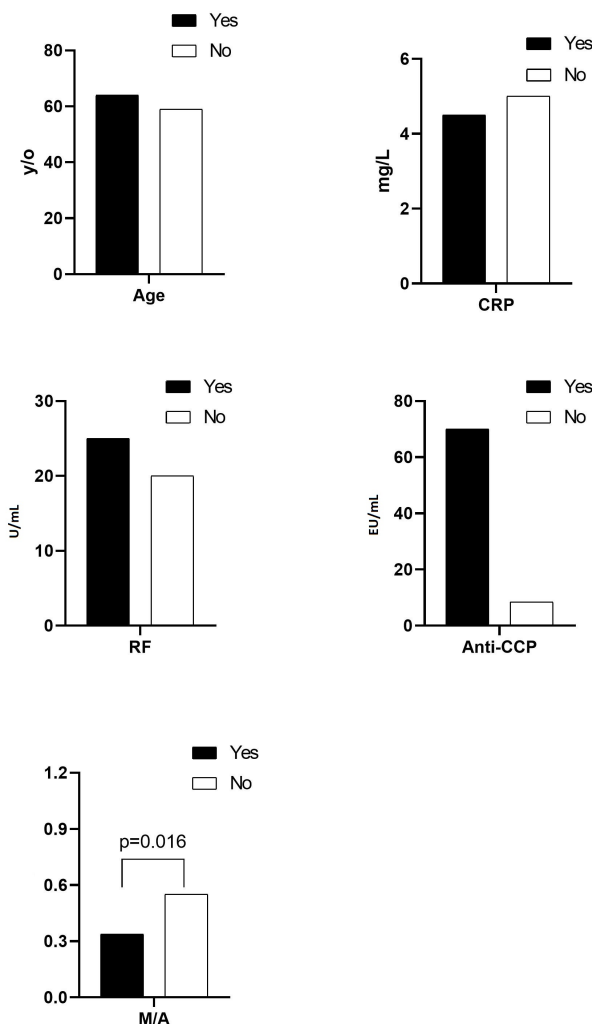


Figure 4. Comparison of groups according to the presence and absence of lung involvement.

When gender was compared, the MA and M/A density ratio were significantly higher in men; however, no significant difference was observed in age ($p = 0.001$, $p = 0.019$, $p = 0.996$, respectively). The findings are summarized in Table 3.

Significance of the model with regression analysis

To further investigate the impact of lung involvement on muscle characteristics, two separate multiple linear regression analyses were conducted. In the first model, muscle area (MA) was entered as the dependent variable, and lung involvement status was coded using two dummy variables: "lung involvement = yes" and "lung involvement = no," with the control group (healthy individuals) as the reference category. The overall model was not statistically significant, $F(2, 184) = 1.315$, $p = 0.271$, indicating that lung involvement status did not significantly predict muscle area. The model explained only 1.4% of the total variance ($R^2 = 0.014$; Adjusted $R^2 = 0.003$). In terms of individual predictors, neither RA patients without lung involvement ($B = -181.61$, $p = 0.117$) nor those with lung involvement ($B = -115.01$, $p = 0.420$) showed a statistically significant difference in muscle area compared to the control group.

In the second model, the M/A density ratio was used as the dependent variable to evaluate changes in muscle quality. Again, the predictor variables were the two dummy-coded lung involvement groups, with the control group as reference. This model was statistically significant, $F(2, 184) = 15.684$, $p < 0.001$, explaining 14.6% of the variance in the M/A density ratio ($R^2 = 0.146$; Adjusted $R^2 = 0.136$), which is a moderate effect size in clinical research.

In terms of regression coefficients, RA patients without lung involvement had significantly lower M/A density ratio than controls ($B = -0.175$, $p = 0.011$), suggesting a moderate decrease in muscle quality. RA patients with lung involvement showed an even more pronounced reduction ($B = -0.462$, $p < 0.001$), highlighting the potential additive or synergistic effect of pulmonary involvement on intramuscular fat infiltration. These findings indicate that while muscle mass (area) may not differ significantly across groups, muscle quality, as reflected by M/A density ratio, is significantly reduced in RA patients, especially in those with pulmonary manifestations. The detailed coefficients and significance values are presented in Table 4.

Correlation analysis between data

The data were analyzed using the Spearman correlation test. MA and M/A density ratio correlation was statistically insignificant ($p = 0.402$).

No association between age and MA ($p = 0.289$). A high negative correlation was observed between the M/A density ratio and age ($p < 0.001$, $\rho = -0.489$).

DISCUSSION

Sarcopenia is a significant comorbidity and extraarticular finding in rheumatoid arthritis (RA), affecting about 25% of individuals with the disease [7]. While lung involvement is the most common extraarticular manifestation of RA [6], the relationship between RA pulmonary involvement and sarcopenia

Table 3. Comparison of muscle area, muscle density, M/A density ratio, and age between genders.

	Muscle Area (mm ²)			Muscle Density (HU)			M/A Density Ratio			Age (year)		
	Median	Minimum	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum
Male	3753	2467	5425	25	-40	55	0.75	0.05	1.43	61	21	86
Female	3280	1090	5300	30	2	52	0.60	-1.60	1.60	59	18	88
p-value	0.001			0.090			0.019			0.996		

p was obtained from the Mann-Whitney U test.

Table 4. Regression analysis predicting muscle area and M/A density ratio.

Dependent variable	Predictor	B	SE	β	t	p
Muscle Area (Adjusted R ² = 0.003, p = 0.271)	(Constant)	3467.61	67.60	--	51.30	<0.001
	Lung involvement = no	-181.61	115.26	-0.12	-1.58	0.117
	Lung involvement = yes	-115.01	142.33	-0.06	-0.81	0.420
M/A Density Ratio (Adjusted R ² = 0.136, p < 0.001)	(Constant)	0.709	0.040	--	17.76	<0.001
	Lung involvement = no	-0.175	0.068	-0.183	-2.57	0.011
	Lung involvement = yes	-0.462	0.084	-0.390	-5.50	<0.001

p-values are based on t-tests within the regression model.

nia has not been widely studied. To the best of our knowledge, this study is the first to investigate this link.

Muscle quality can be evaluated by muscle density obtained from CT, which indicates myocellular lipid content and fatty infiltration [8]. In this study, we assessed muscle mass and quality using CT, a widely accepted technique. To overcome potential density variations caused by imaging parameters, we calculated the ratio of muscle density to aortic density. This approach ensures more precise measurements by eliminating technical differences.

Measuring muscle mass and density via CT is particularly useful in RA because, unlike muscle strength tests, it is not affected by joint inflammation [4]. Our findings support the work of Khoja et al. [9], who demonstrated that skeletal muscle fat infiltration increases in RA patients compared to age-matched controls. We also found that this condition was more pronounced in patients with lung involvement.

Skeletal muscle fat is associated with disease activity and negatively impacts muscle strength, physical performance, and disability, independent of muscle mass [9–11]. Our results align with two studies involving over 100 participants, which found that higher muscle density was associated with less disability (measured by the Health Assessment Questionnaire (HAQ)) and greater physical function (measured by the Short Physical Performance Battery (SPPB)) [11, 12]. Furthermore, studies on non-RA patients have shown that low muscle density is linked to adverse outcomes, including increased weakness, mobility limitations, and a higher risk of hip fractures and hospitalization [11]. The relationship between muscle density and strength is also stronger in RA patients compared to healthy controls [12]. Consistent with the existing literature, our findings suggest that pulmonary involvement in RA may impact muscle quality and contribute to these comorbidities. Although our regression model was statistically significant in predicting the muscle-to-aorta (M/A) density ratio, the ex-

plained variance was modest (Adjusted R² = 0.136). This suggests that other unmeasured factors may contribute to muscle adiposity in RA patients. Important covariates such as BMI, metabolic disorders (e.g., diabetes mellitus, hyperlipidemia), insulin resistance, and physical activity levels were not available due to the study’s retrospective nature. The absence of these variables may have limited the explanatory power of our models. Future prospective studies with comprehensive clinical and metabolic profiling are warranted to better understand the multifactorial nature of muscle fat infiltration in RA.

The accumulation of intramuscular fat in RA patients can be caused by chronic systemic inflammation, physical inactivity, excessive total and visceral adiposity, and the use of medications such as glucocorticoids [11]. Inflammatory cytokines, such as IL-6 and tumor necrosis factor, have been associated with decreased muscle density [13]. A study found that muscle concentrations of IL-6 protein were significantly higher in RA patients compared to controls [14]. When these findings are considered together, it is clear that RA-related inflammation is a significant factor in muscle fat infiltration.

Similar to the study by Baker et al. [15], we observed low muscle density in the RA group. While muscle area was lower in our RA group, this difference was not statistically significant. This supports the findings of Kramer et al., who showed that thigh muscle density, but not area, was associated with less disability and greater physical functioning [11]. In our study, RA and RA-related lung involvement significantly affected muscle density, but not muscle area, suggesting a link to greater disability and reduced physical activity.

Baker et al. found no significant relationship between muscle density and BMI in RA patients, suggesting that BMI may be a limited indicator for assessing muscle density [15]. Consistent with this, our study did not find a difference in subcutaneous adipose tissue area between the groups. However,

our study found a positive correlation between the area of the psoas major muscle and abdominal subcutaneous and visceral fat, which consistent with the literature [16], as well as a negative correlation between the M/A density ratio and subcutaneous fat area. This contradicts the idea that subcutaneous adipose tissue reduces sarcopenia. Consistent with the literature, our study found that the paraspinal muscle area was significantly lower in women [17]. While we found no significant difference in muscle density between men and women, the M/A ratio was significantly lower in women. This suggests that the ratio is a more precise measurement, unaffected by imaging parameters [18]. Unlike cadaveric studies [19], our study found no significant correlation between muscle area and age. This may be because sarcopenia is influenced by factors beyond age, such as inactivity, diseases, and medications. However, consistent with existing literature, we did find a significant negative correlation between M/A density and age.

The absence of a statistical difference in CRP values between the patient and control groups may seem like a negative result at first glance. However, this can be considered a factor increasing the study's statistical power. In this way, more homogeneity was provided for the groups. The fact that there was no significant difference between the groups in terms of CRP values is thought to be due to increased CRP secondary to trauma in the individuals in the control group included in the study [20].

The findings of this study suggest that evaluating muscle density using CT in RA patients, particularly those with pulmonary involvement, may provide a valuable non-invasive tool for early identification of sarcopenia risk. This could guide clinicians in initiating nutritional, physical activity, or pharmacological interventions to mitigate muscle quality deterioration.

Limitations

This study has several limitations. First, due to its retrospective design, BMI, physical activity levels, and metabolic comorbidities could not be included in the analysis. Second, the sample size was limited by the number of available CT scans that met the inclusion criteria. Third, the high proportion of female patients in our study may be explained by the fact that RA is more common in women. However, this may restrict the generalization of the findings to male patients.

CONCLUSION

In this study, unlike the existing literature, we found that RA lung involvement may also lead to increased muscle adiposity. We believe that the primary reason for this is the increase in inflammation. However, immobility and medications used due to lung involvement can also cause this. To better understand this relationship, larger prospective studies investigating the effects of RA-related lung involvement on muscles are needed.

Ethics Committee Approval: This study was approved by Sivas Cumhuriyet University Non-Interventional Clinical Research Ethics Committee (date:19-12-2024, decision number:2024-12/58).

Informed Consent: This retrospective study was approved by the institutional ethics committee. Since the data were collected from existing medical records and anonymized, informed consent was not required.

Peer-review: Externally peer-reviewed.

Conflict of Interest: No author of this paper has a conflict of interest, including specific financial interests, relationships, and affiliations relevant to the subject matter or materials included in this manuscript.

Author Contributions: EG: Conception, Design, Analysis and Interpretation, Data Collection and Preprocessing, Literature Review, Writing, AS: Conception, Materials, Data Collection and Preprocessing, IA: Analysis and Interpretation, Literature Review, Critical Review, SA: Analysis and Interpretation, Literature Review, Critical Review.

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REFERENCES

- Di Matteo A, Bathon JM, Emery P. Rheumatoid arthritis. *Lancet*. 2023;25;402(10416):2019-2033. doi: [10.1016/S0140-6736\(23\)01525-8](https://doi.org/10.1016/S0140-6736(23)01525-8).
- Tekgoz E, Colak S, Ozalp Ates FS, Sonaeren I, Yilmaz S, Cinar M. Sarcopenia in rheumatoid arthritis: Is it a common manifestation? *Int J Rheum Dis*. 2020;23(12):1685-1691. doi: [10.1111/1756-185X.13976](https://doi.org/10.1111/1756-185X.13976).
- Lauren KG, Daniel BG, Stacey VW, et al. Thoracic Manifestations of Rheumatoid Arthritis. *RadioGraphics*. 2021;41(1):32-55. doi: [10.1148/rg.2021200091](https://doi.org/10.1148/rg.2021200091).
- Cruz-Jentoft AJ, Bahat G, Bauer J, et al. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing*. 2019;48(1):16-31. doi: [10.1093/ageing/afy169](https://doi.org/10.1093/ageing/afy169).
- Bennett JL, Pratt AG, Dodds R, Sayer AA, Isaacs JD. Rheumatoid sarcopenia: loss of skeletal muscle strength and mass in rheumatoid arthritis. *Nat Rev Rheumatol*. 2023;19(4):239-251. doi: [10.1038/s41584-023-00921-9](https://doi.org/10.1038/s41584-023-00921-9).
- Kadura S, Raghu G. Rheumatoid arthritis-interstitial lung disease: manifestations and current concepts in pathogenesis and management. *Eur Respir Rev*. 2021;30(160):210011. doi: [10.1183/16000617.0011-2021](https://doi.org/10.1183/16000617.0011-2021).
- Li TH, Chang YS, Liu CW, et al. The prevalence and risk factors of sarcopenia in rheumatoid arthritis patients: A systematic review and meta-regression analysis. *Semin Arthritis Rheum*. 2021;51(1):236-245. doi: [10.1016/j.semarthrit.2020.10.002](https://doi.org/10.1016/j.semarthrit.2020.10.002).
- Shen Y, Luo L, Fu H, et al. Chest computed tomography-derived muscle mass and quality indicators, in-hospital outcomes, and costs in older inpatients. *J Cachexia Sarcopenia Muscle*. 2022;13(2):966-975. doi: [10.1002/jcsm.12948](https://doi.org/10.1002/jcsm.12948).
- Khoja SS, Patterson CG, Goodpaster BH, Delitto A, Piva SR. Skeletal muscle fat in individuals with rheumatoid arthritis compared to healthy adults. *Exp Gerontol*. 2020;129:110768. doi: [10.1016/j.exger.2019.110768](https://doi.org/10.1016/j.exger.2019.110768).
- Andonian BJ, Johannemann A, Hubal MJ, et al. Altered skeletal muscle metabolic pathways, age, systemic inflammation, and low cardiorespiratory fitness associate with improvements in disease activity following high-intensity interval training in persons with rheumatoid arthritis. *Arthritis Res Ther*. 2021;23(1):187. doi: [10.1186/s13075-021-02570-3](https://doi.org/10.1186/s13075-021-02570-3).

11. Kramer HR, Fontaine KR, Bathon JM, Giles JT. Muscle density in rheumatoid arthritis: associations with disease features and functional outcomes. *Arthritis Rheum.* 2012;64(8):2438-50. doi: [10.1002/art.34464](https://doi.org/10.1002/art.34464).
12. Baker JF, Mostoufi-Moab S, Long J, et al. Intramuscular Fat Accumulation and Associations With Body Composition, Strength, and Physical Functioning in Patients With Rheumatoid Arthritis. *Arthritis Care Res (Hoboken).* 2018;70(12):1727-1734. doi: [10.1002/acr.23550](https://doi.org/10.1002/acr.23550).
13. Chang KV, Wu WT, Chen YH, et al. Enhanced serum levels of tumor necrosis factor- α , interleukin-1 β , and -6 in sarcopenia: alleviation through exercise and nutrition intervention. *Aging (Albany NY).* 2023;15(22):13471-13485. doi: [10.18632/aging.205254](https://doi.org/10.18632/aging.205254).
14. Huffman KM, Jessee R, Andonian B, et al. Molecular alterations in skeletal muscle in rheumatoid arthritis are related to disease activity, physical inactivity, and disability. *Arthritis Res Ther.* 2017;19(1):12. doi: [10.1186/s13075-016-1215-7](https://doi.org/10.1186/s13075-016-1215-7).
15. Baker JF, Von Feldt J, Mostoufi-Moab S, et al. Deficits in muscle mass, muscle density, and modified associations with fat in rheumatoid arthritis. *Arthritis Care Res (Hoboken).* 2014;66(11):1612-8. doi: [10.1002/acr.22328](https://doi.org/10.1002/acr.22328).
16. Ni X, Jiao L, Zhang Y, et al. Correlation Between the Distribution of Abdominal, Pericardial and Subcutaneous Fat and Muscle and Age and Gender in a Middle-Aged and Elderly Population. *Diabetes Metab Syndr Obes.* 2021;14:2201-2208. doi: [10.2147/DMSO.S299171](https://doi.org/10.2147/DMSO.S299171).
17. Nuzzo JL. Narrative Review of Sex Differences in Muscle Strength, Endurance, Activation, Size, Fiber Type, and Strength Training Participation Rates, Preferences, Motivations, Injuries, and Neuromuscular Adaptations. *J Strength Cond Res.* 2023;37(2):494-536. doi: [10.1519/JSC.0000000000004329](https://doi.org/10.1519/JSC.0000000000004329).
18. Laskou F, Westbury LD, Fuggle NR, et al. Determinants of muscle density and clinical outcomes: Findings from the Hertfordshire Cohort Study. *Bone.* 2022;164:116521. doi: [10.1016/j.bone.2022.116521](https://doi.org/10.1016/j.bone.2022.116521).
19. Wilkinson DJ, Piasecki M, Atherton PJ. The age-related loss of skeletal muscle mass and function: Measurement and physiology of muscle fibre atrophy and muscle fibre loss in humans. *Ageing Res Rev.* 2018;47:123-132. doi: [10.1016/j.arr.2018.07.005](https://doi.org/10.1016/j.arr.2018.07.005).
20. Nehring, S.M., Goyal, A. and Patel, B.C. (2023). C Reactive Protein. *StatPearls*, Treasure Island (FL). Available from: <https://www.ncbi.nlm.nih.gov/books/NBK441843/>.



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Role of loneliness in the relationship between chronic pain and analgesic use in the elderly

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■ MAIN POINTS

- Loneliness is a significant determinant of chronic pain among the elderly, with higher loneliness levels strongly associated with increased pain scores.
- Frequent analgesic use is independently linked to loneliness, regardless of age, sex, or current pain intensity, suggesting that psychosocial factors drive medication consumption beyond physical symptoms.
- Integrating psychosocial assessment into elderly care is essential, as addressing loneliness and strengthening social support systems may help reduce unnecessary or excessive analgesic use and improve overall pain management.

■ ABSTRACT

Aim: Loneliness is more frequently observed in older adults than in other age groups and is associated with adverse health outcomes. This study aimed to examine the relationship between loneliness, chronic pain, and analgesic use in the geriatric population.

Materials and Methods: This cross-sectional study included 384 elderly subjects who presented to a family medicine outpatient clinic. The University of California, Los Angeles (UCLA) Loneliness Scale for the Elderly and the Geriatric Pain Measure were used to collect data on the variables.

Results: The mean age of the 384 elderly participants included in the study was 74.91±7.12 years (range: 65–91). Of the participants, 52.9% were women and 11.7% were living alone. Higher frequencies of analgesic use were associated with higher pain and loneliness scores ($p<0.001$). A moderate, positive, and statistically significant correlation was found between pain and loneliness scores ($r=0.478$, $p<0.001$). In the multivariate linear regression analysis, each 1-point increase in the loneliness score led to a 1.72-point increase in the pain score. Female sex and chronic diseases were associated with 4.63- and 9.65-point increases in the pain score, respectively. Multinomial logistic regression analysis showed that, independent of age, sex, and pain score, each 1-point increase in the loneliness scale score was associated with a higher likelihood of using analgesics ≥ 15 times/month (OR=1.29), 8–14 (OR = 1.24), and 2–7 (OR = 1.22) times/month. Age and sex did not have any significant effect on monthly analgesic use.

Conclusion: There is a positive relationship between loneliness, chronic pain, and frequent use of analgesics in the elderly subjects. Psychosocial assessment and support in elderly patients may help reduce excessive analgesic consumption.

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■ INTRODUCTION

Loneliness is described as an emotional state arising from the perception that one's social network is narrower or less satisfying than desired. It reflects a sense of deficiency resulting from the discrepancy between the level of emotional satisfaction an individual seeks and the level they actually experience [1-3]. Today, loneliness is widely recognized as a reality of life—an experience encountered by individuals at various points in their lives and to varying degrees [4]. There is no consensus regarding the relationship between loneliness

and age in the literature. Since loneliness is assessed based on subjective perception, it is suggested that it tends to be higher during adolescence—a period often marked by emotional turbulence—declines in early and middle adulthood and then increases again in older age, thus presenting a U-shaped trajectory across the lifespan [4-6]. Loneliness causes various physical, psychological, and cognitive problems in individuals. It is a significant factor contributing to deteriorated health and reduced health related quality of life (HRQoL). Given that loneliness is observed more frequently among the

elderly—and that additional challenges faced by this population may worsen the situation—addressing loneliness and implementing effective intervention strategies becomes a crucial necessity. Disorders caused by loneliness can also negatively affect physical health [7]. During the aging process, a decline in the function of bodily systems, reduced organ reserves, and weakened adaptation to the environment are observed. These changes increase the susceptibility to diseases and injuries, which in turn lead to higher rates of medication use [8]. Chronic pain is one of the leading causes of diminished HQoL in the geriatric population. The elderly often resort to over-the-counter analgesics without consulting a physician to manage chronic pain [9]. Investigating the impact of loneliness on chronic pain and its influence on analgesic consumption among older adults may offer valuable insights into this issue. This study aimed to examine the relationship between loneliness, chronic pain, and analgesic use in individuals within the geriatric age group.

MATERIALS AND METHODS

Study design, sample, and ethics

This cross-sectional study was conducted in 2025 among patients aged 65 years who presented to the Family Medicine Outpatient Clinic of the Faculty of Medicine, Izzet Baysal Training and Research Hospital, Bolu Abant Izzet Baysal University. The required sample size was calculated using the formula $n = [DEFF \times N \times p(1-p)] / [(d^2 / Z^2_{1-\alpha/2} \times (N-1)) + p(1-p)]$ * with 80% power, 95% confidence interval, $d = 50\%$, and $DEFF = 1$. Accordingly, the minimum sample size was determined to be 384 individuals. Participants were recruited through convenience sampling. Data were collected through face-to-face interviews with patients who presented to the Family Medicine Clinic between April and June 2025. All participants were informed about the study procedures and provided written informed consent. After completing the data collection tools, the researcher reviewed the responses and provided necessary clarifications. Ethical approval for the study was obtained from the Ethics Committee of Bolu Abant Izzet Baysal University Faculty of Medicine, Izzet Baysal Training and Research Hospital (Decision No: 2025/150, Date: 08.04.2025).

Exclusion criteria

- Participants with cognitive impairments (e.g., advanced dementia, delirium) or neurological conditions (e.g., aphasia) that prevent them from understanding or responding to the questionnaire.
- Severe hearing impairment or speech disability precluding verbal or written communication despite assistance.
- Individuals younger than 65 years.

Data collection

Data were collected from 384 patients who visited the Family Medicine Outpatient Clinic of Bolu Abant Izzet Baysal University between April and June 2025. The data collection form consisted of the following three sections:

- Demographic information (age, gender, marital status, and analgesic use).
- UCLA Loneliness Scale for the Elderly.
- Geriatric Pain Measure.

The University of California, Los Angeles Loneliness Scale for the Elderly

Originally developed in 1985 by De Jong Gierveld and Kamphuis to assess emotional loneliness and revised in 1999 by Van Tilburg and De Jong Gierveld, the UCLA Loneliness Scale for the Elderly is based on the cognitive-behavioral framework [10]. Akgül et al. conducted the Turkish validity and reliability study in 2015. The Cronbach's alpha of the scale is $\alpha = .85$. The test-retest result of the scale is $r = .93$ [11]. The scale employs a 3-point Likert-type response format (0 = Yes, 1 = Maybe, 2 = No) to determine the extent to which each item describes the experience of the respondents. Respondents were asked to select the option that best reflected their current state. The scale consists of 11 items, of which 6 are positively worded and 5 are negatively worded. Items 1, 4, 7, 8, and 11 are scored as follows: 0 = Yes, 1 = Maybe, and 2 = No. Items 2, 3, 5, 6, 9, and 10 are reverse-coded: 2 = Yes, 1 = Maybe, and 0 = No. The total score ranges from 0 to 22, with higher scores indicating higher loneliness levels.

Geriatric Pain Measure

The Geriatric Pain Measure (GPM) was developed by Bruce A in 2000. Ferrell et al. comprehensively assessed the functional, psychological, and social effects of pain in older individuals [12]. The Turkish adaptation and validation study was conducted by Dursun et al. in 2017, yielding a Cronbach's alpha of 0.85 [13]. Item content and cultural adaptations were based on expert opinions. The scale consists of five dimensions: pain-related withdrawal (Items 6, 17, 18, 19, 20, 21, and 24), pain intensity (Items 1, 2, 3, 4, 5, 22, and 23), pain with movement (Items 9, 10, 11, and 12), pain with strenuous activities (Items 8, 13, and 14), and pain with other activities (Items 7, 15, 16, 17, and 22). Two items (17 and 22) are included in more than one subscale. The scale also contains three open-ended questions about pain. Of the 24 items, 22 are scored dichotomously (Yes/No), while the remaining 2 items are rated on a 0-10 scale. The total score is calculated by summing the number of "Yes" responses, resulting in a raw score ranging from 0 to 42. Each item score is then multiplied by 2.38 to convert it to a scale of 0–100. The final score of the Geriatric Pain Measure is calculated based on this 0-100 scale. Scores between 0 and 30 indicate mild pain, scores between

31 and 69 indicate moderate pain, and scores of 70 or above indicate severe pain. The Geriatric Pain Measure evaluates the manner in which patients describe their pain and assesses the physical, emotional, cognitive, and behavioral responses associated with pain [12,13].

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 21.0 (Armonk, NY: IBM Corp.) and JAMOVI 2.6.17 version software. The Kolmogorov–Smirnov test was used to assess normality of the test. Multivariable linear regression, one-way analysis of variance, multinomial logistic regression, and Pearson correlation analyses were conducted. The Bonferroni test was used in the post-hoc tests. A p-value of <0.05 was considered statistically significant.

RESULTS

The mean age of the 384 older adults included in the study was 74.91 ± 7.12 years (min: 65, max: 91). Among the participants, 52.9% were female, 30.7% were single or widowed, and 81.3% had at least one chronic illness. Regarding living arrangements, 11.7% of the older adults lived alone, 65.4% lived with their spouse, and 20.3% lived with their children.

Table 1 presents the sociodemographic characteristics of the participants.

When examining the correlation between the scores obtained from the pain and loneliness scales among elderly subjects was statistically significant and moderately positive (r = 0.478) (Figure 1).

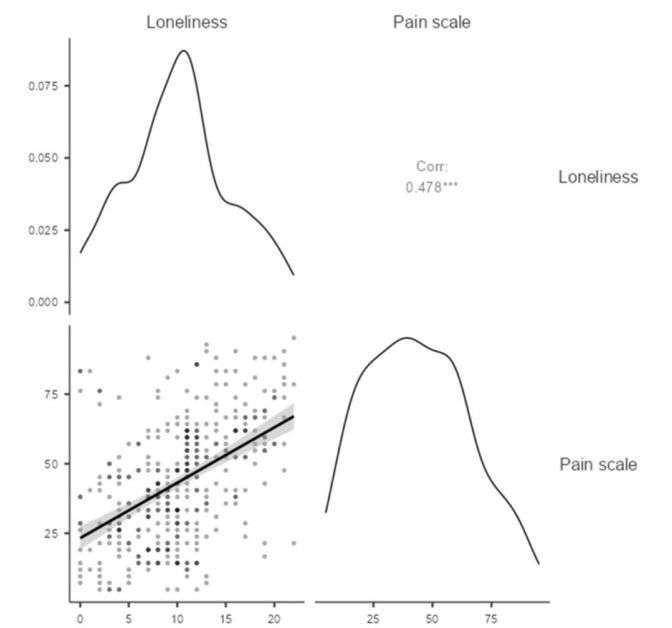


Figure 1. Correlation analysis.

The average score of the participants on the loneliness scale was 10.13±5.20 (min: 0-max: 22), and the average score on the geriatric pain scale was 43.42±21.64 (min: 4.76-max:

Table 1. Sociodemographic characteristics of participants.

		n	%
Sex	Male	181	47.1
	Female	203	52.9
Marital Status	Married	266	69.3
	Single	10	2.6
	Widowed	108	28.1
Educational Level	Literate (no formal education)	155	40.4
	Primary School	127	33.1
	Secondary School	50	13.0
	High School	36	9.4
	University	13	3.4
	Master's Degree	3	.8
Income Status	Income is less than expenses	105	27.3
	Income equals expenses	213	55.5
	Income is more than expenses	66	17.2
Presence of Chronic Illity	Yes	312	81.3
	No	72	18.8
Cardiovascular Disease	Yes	236	61.5
	No	148	38.5
Endocrine Disease	Yes	97	25.3
	No	286	74.7
Neurological Disease	Yes	61	15.9
	No	323	84.1
Psychiatric Disorder	Yes	25	6.5
	No	359	93.5
Respiratory Disease	Yes	31	8.1
	No	353	91.9
Cancer	Yes	11	2.9
	No	373	97.1
Musculoskeletal Disorder	Yes	38	9.9
	No	346	90.1
Urological Disease	Yes	36	9.4
	No	348	90.6
Renal Failure	Yes	15	3.9
	No	369	96.1
Other Diseases	Yes	33	8.6
	No	351	91.4
Living Arrangement	With spouse	251	65.4
	With children	78	20.3
	With relatives or friends	10	2.6
	Alone	45	11.7
Has a Caregiver	Yes	27	7.0
	No	357	93.0
Frequency of analgesic use	15 times or more per month	82	21.4
	8-14 times per month	100	26.0
	2-7 times per month	151	39.3
	Once a month or less	51	13.3

Note: The total number of chronic diseases exceeded 385 because some participants had more than one chronic condition.

95.20). A statistically significant difference was observed when the scores from the loneliness and pain scales were compared according to the frequency of analgesic use among older adults. An increased frequency of analgesic use was associated

Table 2. Comparison of loneliness and pain scores at analgesic use frequency.

	Loneliness	Pain
	Mean±SD	Mean±SD
15 times or more per month ^{a,b,c}	13.39±4.93	62.02±17.86
8-14 times per month ^a	10.88±4.38	47.71±16.93
2-7 times per month ^b	9.43±4.63	35.79±19.36
Once a month or less ^c	5.51±4.87	27.67±18.55
p	<0.001	<0.001

Different superscript letters (a, b, c) indicate significant differences between groups according to Bonferroni post hoc test ($p < 0.05$).

with higher scores on both scales. People who used 15 or more painkillers per month had statistically higher scores on both scales (Table 2).

Multivariable linear regression analysis was conducted to predict pain scores among older adults, and the model was found to be statistically significant ($p < 0.001$). The coefficient of determination for the model was $R^2 = 0.283$. The dependent variable in the model was the total score from the pain scale, whereas the independent variables were the loneliness scale score, age, gender (reference: male), and presence of chronic illness (reference: no). Among the variables included in the model, loneliness score, gender, and presence of chronic illness significantly contributed to the prediction. It was found that A one-point increase in the loneliness scale score was associated with a 1.72-point increase in the pain score. Female sex was associated with a 4.63-point increase in the pain score compared to male sex. Having a chronic illness was associated with a 9.65-point increase in the pain score compared with those without a chronic illness (Table 3).

A multinomial logistic regression analysis was performed to estimate the frequency of monthly analgesic use. The results indicated that the model was significant (*model fitting information*, $*p^* < 0.001$), exhibited adequate data fit (deviance, $*p^* = 0.393$), and demonstrated sufficient goodness-of-fit (*Cox and Snell* = 0.396). Independent of age, sex, and pain score, each one-unit increase in the loneliness scale raised the risk of using analgesics 15 or more times per month by 1.29-fold, the risk of using analgesics 8–14 times per month by 1.24-fold, and the risk of using analgesics 2–7 times per month by 1.22-fold, compared to the group using one or fewer analgesics per month. Similarly, a one-unit increase in pain score increased the risk of using analgesics 15 or more times per month by 1.073-fold and the risk of using analgesics 8–14 times per month by 1.035-fold, again relative to the group using one or fewer analgesics per month. Additionally, the presence of chronic disease elevated the risk of using analgesics 15 or more times per month by 8.51-fold, the risk of using analgesics 8–14 times per month by 9.95-fold, and the risk of using analgesics 2–7 times per month by 2.77-fold, compared to the group using one or fewer analgesics per month. Finally, sex and age had no significant effect on monthly anal-

gesic use (Table 4).

DISCUSSION

The high prevalence of multiple chronic conditions (comorbidities) among older adults is a primary factor contributing to increased polypharmacy. Pietraszek et al. reported that analgesics ranked second among the most frequently used medications in elderly subjects, with a usage rate of 46.0% [14]. This study investigated the relationships among loneliness, chronic pain, and analgesic use in the elderly subjects. Our findings demonstrated a moderate, positive, and significant correlation between loneliness and pain levels. Each one-unit increase in the loneliness scale score corresponded to a 1.72-unit increase in the pain score. The significance of this finding, coupled with the lack of prior studies directly comparing these two conditions (loneliness and pain), underscores the importance of this research and highlights the need for further detailed investigations. Independent of age, sex, and pain score, each unit increase in the loneliness score increased the risk of using analgesics ≥ 15 times per month by 1.29 times; 8–14 times per month by 1.24 times; and 2–7 times per month by 1.22 times. In a study by Vyas et al. involving 15,302 older adults, the prevalence of loneliness was 13.7%. Those living alone had higher rates of opioid (odds ratio [OR] = 1.61) and benzodiazepine (OR = 1.66; 95% confidence interval [CI]: 1.21–2.28) use, whereas no significant difference was observed for non-opioid analgesics (OR = 1.05; 95% CI: 0.92–1.19). Additionally, loneliness was significantly associated with intermittent medication use (OR = 1.27) [15]. Various studies in the literature have also reported that feelings of loneliness in this age group are particularly linked to the regular use of opioids and benzodiazepines [16,17]. International studies present mixed findings: for instance, in the United States, living alone was not significantly associated with increased use of these medications [18], whereas a positive correlation was observed between loneliness and analgesic use frequency in Germany [19]. However, these studies generally report the relationship mainly for opioid analgesics, with nonopioid analgesics showing less clear associations. Our study did not differentiate analgesic use by opioid versus non-opioid categories; however, non-opioid analgesics are presumed to be more commonly used given that health policies in our country discourage opioid use unless strictly necessary. Therefore, we believe that non-opioid medications also contributed to the significant findings of our study. Differences in findings across studies may reflect varying national drug use policies. Im et al. analyzed the relationship between loneliness and polypharmacy by sex in a study including 2,348 older adults, revealing a stronger association in women. The prevalence of polypharmacy was highest in the severe loneliness group among women (44.1%) and men (42.5%). Moreover, antidepressant use was reported to be more common in lonely women [20]. Sönmez et al. also found that elderly individuals with polypharmacy had

Table 3. Multivariate linear regression analysis for predicting pain scores.

Predictor	Estimate	SE	t	p	Collinearity Statistics	
					VIF	Tolerance
Intercept ^a	-0.0842	10.001	-0.00842	0.993		
Loneliness score	1.7201	0.191	8.98785	<.001	1.12	0.892
Age	0.2107	0.140	1.50156	0.134	1.13	0.886
Sex						
Female--Male	4.6339	1.911	2.42494	0.016	1.03	0.971
Chronic Disease						
Yes--No	9.6565	2.502	3.86001	<.001	1.08	0.927

Table 4. Multinomial logistic regression analysis for estimating the frequency of analgesic use.

Frequency of analgesic use		95 percent CI for O.R.				
		B	p	O.R.	Lower Bound	Upper Bound
15 times or more per month	Intercept	-11.331	<0.001			
	Age	.063	0.067	1.065	.996	1.139
	Loneliness	.262	<0.001	1.299	1.168	1.446
	Pain score	.071	<0.001	1.073	1.046	1.102
	[Sex=female]	-.220	.629	.803	.329	1.959
	[Sex=male, ref]	0 ^b	.	.	.	.
	[Chronic disease=yes]	2.142	0.001	8.515	2.348	30.883
	[Chronic disease=no, ref]	0 ^b	.	.	.	.
8-14 times per month	Intercept	-6.367	0.008			
	Age	.031	0.327	1.032	.969	1.099
	Loneliness	.218	<0.001	1.244	1.126	1.374
	Pain score	.035	.003	1.035	1.012	1.059
	[Sex=female]	-.119	.771	.888	.399	1.977
	[Sex=male, ref]	0 ^b	.	.	.	.
	[Chronic disease=yes]	2.298	<0.001	9.952	3.380	29.303
	[Chronic disease=no, ref]	0 ^b	.	.	.	.
2-7 times per month	Intercept	-2.008	.348			
	Age	.011	.709	1.011	.954	1.071
	Loneliness	.206	<0.001	1.229	1.120	1.347
	Pain score	.006	.566	1.006	.985	1.027
	[Sex=female]	-.087	.812	.917	.448	1.876
	[Sex=male, ref]	0 ^b	.	.	.	.
	[Chronic disease=yes]	1.022	.007	2.779	1.319	5.857
	[Chronic disease=no, ref]	0 ^b	.	.	.	.

a. The reference category is: Once a month or less. b. 0^b indicates the reference category used in the multinomial logistic regression model.

higher loneliness levels, with loneliness being more prevalent in women [21]. In contrast, our study found that the relationship between loneliness and analgesic use was independent of sex. Because our study assessed only analgesic use, the lack of a sex effect might be due to this narrower scope. Svensson et al. investigated the impact of polypharmacy on loneliness and reported that individuals with polypharmacy had a significantly higher likelihood of developing loneliness than those without polypharmacy (odds ratio [OR]: 1.37). The probability of loneliness was 28% and 35% in those without and with polypharmacy, respectively, indicating that polypharmacy increases the risk of loneliness and social isolation in older adults [22]. These findings support the existence of a relationship between polypharmacy and loneliness. While aging is an important risk factor for loneliness, polypharmacy is more com-

mon in older age. Although age could be a confounding factor, our results indicate that loneliness is strongly associated with increased use of analgesics regardless of age. Thus, the hypothesis that polypharmacy triggers loneliness is less clinically plausible than loneliness influencing medication use. Higher frequency of analgesic use correlates with higher loneliness scores. Loneliness may induce prolonged use of medications, including those with high addiction potential, among older adults individuals. Clinicians should consider loneliness as a significant factor when evaluating elderly patients and exercise caution when prescribing medications. Comprehensive research is needed to elucidate the effect of loneliness on medication-taking behaviors, with particular emphasis on psychosocial factors.

Limitations

This study has several limitations. First, the regression models classified chronic diseases as a single variable. This grouping included musculoskeletal conditions, which may independently increase the use of analgesics and could have acted as a confounding factor in the analysis. Second, the data were collected between April and June 2025, which may not capture seasonal variations in loneliness and pain—both of which may be more pronounced during the winter months. Finally, we did not collect data on mood status and polypharmacy, which represents an additional limitation and suggests the need for further, comprehensive studies on this topic

CONCLUSION

This study revealed a significant positive association between loneliness and chronic pain. The data indicate that as loneliness levels increase, self-reported pain scores also rise. Moreover, loneliness increases the frequency of analgesic use independently, regardless of age, sex, and current pain intensity. These results suggest that loneliness is not merely a psychological condition but may be a critical determinant in the management of chronic pain. Elevated loneliness levels may lead to more frequent and potentially unnecessary analgesic use in older adults coping with chronic pain. Therefore, assessing loneliness and strengthening social support mechanisms in elderly populations may mitigate the indirect effects of loneliness and promote more rational pain management. Integrating psychosocial evaluations into standard clinical practice is crucial for preventing inappropriate medication use. In conclusion, loneliness, chronic pain, and excessive analgesic use have a multifaceted and complex relationship. Prospective studies with large sample sizes are needed to better understand the underlying mechanisms of these associations. Such research can provide valuable insights that may guide both clinical practice and health policy development.

Ethics Committee Approval: This study was approved by the Ethics Committee of Bolu Abant İzzet Baysal University Faculty of Medicine, İzzet Baysal Training and Research Hospital (Decision No: 2025/150, Date: 08.04.2025).

Informed Consent: Written informed consent was obtained from all participants included in the study.

Peer-review: Externally peer-reviewed.

Conflict of Interest: The authors declare that they have no conflict of interest.

Author Contributions: A.T.Ö; Conception, Design, Fundings, Materials, Analysis and/or Interpretation, Literature Review, Writing. Y.K.; Conception, Design, Supervision, Fundings, Materials, Critical Review. M.B.; Conception, Design, Supervision, Fundings, Materials. M.K.; Supervision, Data Collection and/or Processing, Literature Review Writing, Critical Review.

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REFERENCES

1. Peplau LA, Perlman D. Perspectives on loneliness. In L. A. Peplau and D. Perlman (Eds.), *Loneliness: A sourcebook of current theory, research and therapy*. New York: Wiley; 1982. pp. 1-18.
2. Hawkey LC, Cacioppo JT. Loneliness Matters: A Theoretical and Empirical Review of Consequences and Mechanisms. *Ann Behav Med*. 2010;40(2):218-27. doi: [10.1007/s12160-010-9210-8](https://doi.org/10.1007/s12160-010-9210-8).
3. Heinrich LM, Gullone E. The clinical significance of loneliness: a literature review. *Clin Psychol Rev*. 2006;26(6): 695-718. doi: [10.1016/j.cpr.2006.04.002](https://doi.org/10.1016/j.cpr.2006.04.002).
4. Luanaigh CÖ, Lawlor BA. Loneliness and health of older people. *Int J Geriatr Psychiatry*. 2008;23(12):1213-21. doi: [10.1002/gps.2054](https://doi.org/10.1002/gps.2054).
5. Perlman D, Peplau LA. Loneliness research: A survey of empirical findings In: Peplau LA, editor. *Prevention of the harmful consequences of severe and persistent loneliness*. New York: Wiley; 1984. p. 13-46.
6. Dykstra PA. Older adult loneliness: myths and realities. *Eur J Aging*. 2009;6(2):91-100. doi: [10.1007/s10433-009-0110-3](https://doi.org/10.1007/s10433-009-0110-3).
7. Çam C, Atay E, Işıklı B. Yaşlılarda yalnızlık ve yaşam kalitesi. *Türk Dünyası Uyg Arş Mer Halk Sağ Dergi*. 2018;3(2):50-67.
8. Gülhan R. Yaşlılarda akılcı ilaç kullanımı. *Okmeydanı Tıp Dergisi*. 2013;29(Suppl 2):1001. doi: [10.5222/otd.suppl.2013.099](https://doi.org/10.5222/otd.suppl.2013.099).
9. Barkin RL, Beckerman M, Blum SL, Clark FM, Koh EK, Wu DS. Should nonsteroidal anti-inflammatory drugs (NSAIDs) be prescribed to the older adult? *Drugs Aging*. 2010;27(10):775-89. doi: [10.2165/11539430-000000000-00000](https://doi.org/10.2165/11539430-000000000-00000).
10. De Jong-Gierveld J, Van Tilburg TG. *Manual of the Loneliness Scale*. Vrije Universiteit Amsterdam; 1999.
11. Akgül H, Yeşilyaprak B. “Yaşlılar için yalnızlık ölçeği” nin Türk kültürüne uyarlaması: geçerlilik ve güvenilirlik çalışması. *Yaşlı Sorunları Araştırma Dergisi*. 2015;8(1):34-45.
12. Ferrell BA, Stein WM, Beck JC. The Geriatric Pain Measure: Validity, Reliability, and Factor Analysis. *J Am Geriatr Soc*. 2000;48(12):1669-73. doi: [10.1111/j.1532-5415.2000.tb03881.x](https://doi.org/10.1111/j.1532-5415.2000.tb03881.x).
13. Dursun G, Bektas H. Cultural Validation and Reliability of the Turkish Version of the Geriatric Pain Measure *Pain Pract*. 2017;17(4):50513. doi: [10.1111/papr.12473](https://doi.org/10.1111/papr.12473).
14. Pietraszek A, Agrawal S, Drózd M et al. Sociodemographic and health-related factors influencing drug intake in the elderly population. *Int J Environ Res Public Health*. 2022;19(14):8766. doi: [10.3390/ijerph19148766](https://doi.org/10.3390/ijerph19148766).
15. Vyas MV, watt JA, Yu AYY, Straus SE, Kapral MK. The association between loneliness and medication use in older adults. *Age Aging*. 2021;50(2):587-91. doi: [10.1093/ageing/afaa177](https://doi.org/10.1093/ageing/afaa177).
16. Kuerbis A., Sacco, P., Blazer, D.G., Moore, A.A. Substance use among older adults. *Clin Geriatr Med*. 2014;30(3):629-54. doi: [10.1016/j.cger.2014.04.008](https://doi.org/10.1016/j.cger.2014.04.008).
17. Segrin C, McNelis M, Pavlich CA. Indirect effects of loneliness on substance use through stress. *Health Commun*. 2018;33(5):513-518. doi: [10.1080/10410236.2016.1278507](https://doi.org/10.1080/10410236.2016.1278507).
18. Day BF, Rosenthal GL. Social isolation proxy variables and prescription opioid and benzodiazepine misuse among older adults in the U.S.: a cross-sectional analysis of data from the National Survey on Drug Use and Health, 2015–2017. *Drug Alcohol Depend*. 2019;204:107518. doi: [10.1016/j.drugalcdep.2019.06.020](https://doi.org/10.1016/j.drugalcdep.2019.06.020).
19. Boehlen F, Herzog W, Quinzler R et al. Elderly loneliness is associated with the use of psychotropic drugs. *Int J Geriatr Psychiatry*. 2015;30(9):957-64. doi: [10.1002/gps.4246](https://doi.org/10.1002/gps.4246).
20. Im JHB, Bronskill SE, Strauss R, et al. Sex-based differences in the association between loneliness and polypharmacy among older adults in Ontario, Canada. *Journal of Occupational Therapy. J Am Geriatr Soc*. 2023;71(10):3099-3109. doi: [10.1111/jgs.18477](https://doi.org/10.1111/jgs.18477).

21. Sönmez Sari E, Çetinkaya Özdemir S, Semerci Çakmak V, Kurt, F. The effect of sociodemographic characteristics and polypharmacy on loneliness and social isolation in community-dwelling older adults. *Geriatr Nurs.* 2024;60:541-547. doi: [10.1016/j.gerinurse.2024.10.023](https://doi.org/10.1016/j.gerinurse.2024.10.023).
22. Svensson, M., Ekström H, Elmståhl S, Rosso, A. Association of polypharmacy with loneliness and social isolation among older adults. *Arch Gerontol Geriatr.* 2024;116:105158. doi: [10.1016/j.archger.2023.105158](https://doi.org/10.1016/j.archger.2023.105158).



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Evaluation of risk factors for morbidity and mortality in intensive care unit readmissions

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■ MAIN POINTS

- Unplanned ICU readmission was strongly associated with increased in-hospital mortality, with a mortality rate of 66.7% among readmitted patients compared to 0% in controls.
- Independent predictors of mortality included higher APACHE II scores, lower serum albumin levels, and the presence of nosocomial infections.
- Dementia/Alzheimer's disease and cerebrovascular disease were significantly more common among readmitted patients, highlighting vulnerable subgroups.
- Identifying high-risk patients through clinical and laboratory parameters may guide early interventions, improve discharge planning, and reduce ICU readmission-related morbidity and mortality.

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■ ABSTRACT

Aim: Unplanned readmissions to the intensive care unit (ICU) are associated with poor clinical outcomes, prolonged hospital stays, and increased healthcare costs. Identifying the clinical and laboratory predictors of ICU readmission and its impact on in-hospital mortality remains critical for improving patient safety and optimizing utilization of the resources. This study aimed to investigate the factors associated with ICU readmission and evaluate its relationship with mortality in a tertiary-care hospital setting.

Materials and Methods: In this retrospective cohort study, data of 1347 patients followed in Pulmonary ICU between 2016 and 2024 were retrospectively evaluated. Data of 153 patients (75 patients readmitted and 78 control cases selected randomly who were not readmitted during the same hospitalization) were analyzed. The two groups -those with and without ICU readmission- were compared in terms of demographic characteristics, clinical parameters, comorbidities, nutritional status, and laboratory findings. Logistic regression analysis was used to identify predictors of mortality.

Results: Readmitted patients were older ($p=0.001$), had lower Glasgow Coma Scores ($p<0.001$), higher APACHE II scores ($p<0.001$), and longer ICU and hospital stays ($p=0.002$, $p=0.006$ respectively). They also required more vasopressors ($p=0.004$), mechanical ventilation ($p=0.001$), and sedation ($p<0.001$). Nosocomial infections were more frequent in this group ($p<0.001$). Univariate regression analysis revealed that ICU readmission, low serum albumin, nosocomial infections, use of vasopressors, and comorbidities such as dementia and cerebrovascular disease were significantly associated with mortality ($p<0.05$). In the Backward Wald model, albumin during hospitalization, nosocomial infection and APACHE II scores were independent risk factors for mortality ($p<0.05$).

Conclusion: ICU readmission is strongly associated with adverse clinical outcomes and increased in-hospital mortality. Identifying high-risk patients based on clinical and laboratory parameters—such as low serum albumin levels, presence of nosocomial infections, use of vasopressors, and comorbidities like dementia/Alzheimer's disease—may facilitate early interventions and improve patient prognosis.

Keywords: Readmission, ICU care, Risk factors, Comorbidity, Mortality, Morbidity

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■ INTRODUCTION

Intensive care units (ICUs) are high-cost hospital settings where critical illnesses and complications are managed using advanced technologies and specialized staff. Their rational use is essential due to the substantial financial burden on hospitals and the healthcare system [1].

ICU readmission refers to the return of a patient to the ICU

during the same hospitalization and is associated with adverse outcomes and increased health care costs [2]. Additionally, ICU readmissions result in longer hospital and ICU stays and are linked to higher morbidity and mortality rates [3-6]. ICU readmissions are also used as indicators of care quality. Although many scoring systems have been developed to minimize these readmissions, an optimal model has not yet been

established [7].

In intensive care units, the decision to transfer to the ward is made by intensivist. Despite the growing interest in decision support tools, the timing of discharge remains largely based on clinical judgment. This daily, complex process challenges both the ICU team and the attending physician [8]. Providing information about reasons for ICU readmission is important for preventing morbidity and mortality in critically ill patients and reducing the clinician burden.

ICU readmissions, adverse events in patients, and their impact on mortality are primarily studied in and supported by scientific articles [2]. However, such data are lacking in with limited resources. Investigating this issue in our tertiary care hospital, which treats many patients with chest diseases, is crucial for establishing data for Turkey. Therefore, this study aimed to identify risk factors for mortality and morbidity among patients readmitted to the pulmonary ICU.

MATERIALS AND METHODS

Study design

This retrospective observational study was conducted at Istanbul Sultan Abdulhamid Han Training and Research Hospital. This study aimed to investigate the clinical, demographic, and laboratory factors associated with ICU readmission in patients in the pulmonary ICU between 2016 and 2024.

Study population

A total of 1,347 patients who were admitted to the Pulmonary ICU between 2016 and 2024 were screened. Among them, 75 patients who were readmitted to the ICU within 48 h after discharge during the same hospitalization were identified as the readmission group. Additionally, 78 patients who were hospitalized in the pulmonary ICU only once during the same period were randomly selected and defined as the non-readmission group. Random sampling was performed to ensure a balanced sample size between the readmission and control groups using IBM SPSS Statistics version 30. Under the “Select Cases” module, the “Random sample of cases” option was utilized, and a total of $n=78$ non-readmitted patients were randomly selected from the dataset and included in the analysis as the control group. This method was chosen to minimize selection bias and enhance comparability between the groups.

Only patients aged 18 years were included in the study. Although patients with conditions such as dementia or Alzheimer’s disease requiring palliative care were included, none of these patients were admitted from dedicated palliative care centers.

Patients who were admitted to other ICUs within the same hospital or to ICUs in other hospitals were excluded from the study. In addition, patients who were discharged upon their request and those with terminal-stage malignancies were excluded from the study.

Study parameters

Age, gender, comorbidities such as diabetes mellitus (DM), coronary artery disease (CAD), chronic obstructive pulmonary disease (COPD), congestive heart failure (CHF), arrhythmias, pulmonary and extrapulmonary malignancies, dementia/Alzheimer’s disease, and cerebrovascular diseases (CVD), demographic and clinical characteristics of the patients, and laboratory findings (hemogram, blood biochemistry, inflammatory markers, and blood gas) at the time of admission and before discharge from the ICU to the ward were recorded. In addition, the Glasgow coma scale (GCS), APACHE II score, need for mechanical ventilation (MV), non-invasive mechanical ventilation (NIMV), vasopressor therapy, sedation, and dialysis during ICU stay were noted in patients admitted to the ICU. The number of hospital and ICU hospitalization days, number of MV and NIMV days, nutritional characteristics, and nosocomial infections were also recorded. The Prognostic Nutritional Index (PNI), which determines the nutritional status of patients, and the PIV, which reflects the inflammatory status of the patients, were also checked during hospitalization. Patients who were readmitted to the ICU ($n = 75$) and those who were not readmitted ($n = 78$) were compared in terms of the mentioned parameters.

Statistical analysis

The data obtained in the study were analyzed using the Statistical Package for the Social Sciences software. Continuous variables were presented as mean $\pm$ standard deviation (mean $\pm$ SD), and categorical data were presented as the number of affected individuals and percentage of the study population (%). For group comparisons, an independent samples t-test was used for parametric data, and a Chi-square test or Fisher’s exact test was used for categorical variables. The Mann–Whitney U test was preferred for comparing variables that were not normally distributed. Univariate and multivariate logistic regression analyses were performed to determine the factors associated with mortality, and the effects of significant variables were reported with odds ratios (ORs), 95% confidence intervals (CIs), and p-values. The significance level was accepted as any p-value being < 0.05 . No bootstrap resampling was performed in the statistical analysis.

Multivariable logistic regression analysis was performed using the backward Wald method. The relationships among independent variables were assessed using the variance inflation factor (VIF) values calculated under linear regression, and all VIF values were below 5, indicating no significant multicollinearity. Model calibration was evaluated using the Hosmer-Lemeshow goodness-of-fit test, which demonstrated adequate fit ($p>0.05$). The discriminative ability of the model was assessed using a receiver operating characteristic (ROC) curve, and the area under the curve (AUC) was calculated.

The sample size was estimated using G*Power 3.1 software, assuming a 6% ICU readmission rate of 6%, a statistical power

of 90%, a significance level of 5%, and three independent variables with a medium effect size (Cohen's $f^2 = 0.15$). Based on these parameters, a minimum of 1,243 patients and at least 75 ICU readmission events were required to achieve statistical significance.

■ RESULTS

General characteristics of the study population

The study included 153 patients, of whom 58.82% were female ($n = 90$) and 41.18% were male ($n = 63$). The mean age was 71.43 ± 14.32 years. Hospitalization to the ICU occurred in 54.9% of patients from the emergency department and 45.1% from hospital wards. The most common admission diagnosis was pneumonia (33.3%), followed by COPD exacerbation (20.9%) and pulmonary embolism (11.1%).

The need for vasopressors was observed in 35.95% of patients, MV in 18.3%, NIMV in 56.21%, and oxygen with HFNC in 16.45%. The following comorbidities were present: DM (31.37%), COPD (43.79%), HT (58.17%), CHF (29.41%), extrapulmonary malignancy (14.38%), lung cancer (9.15%), dementia/AD (12.42%), and CVD (10.46%). The comorbidity rate was high, with 94.12% of patients having at least one comorbid disease.

After the initial treatment, 70.59% of the patients was transferred to the ward within working hours. The most common reason for ICU readmission was hypoxic respiratory failure (64%), followed by hypotension/shock (22.7%). The mean length of ICU stay was 7.97 ± 8.29 days, and the total hospital stay was 1.92 ± 4.01 days. Patients were readmitted to the ICU after a mean of 8.21 ± 8.84 days.

Demographic, clinical, and laboratory findings of patients with and without icu readmission

The mean age was significantly higher in the readmitted group (75.25 ± 12.42 vs. 67.76 ± 15.13 years; $p=0.001$). The GCS scores measured at initial hospitalization and during ICU discharge were significantly lower in the readmitted group ($p<0.001$). In addition, patients in this group had higher APACHE II scores at the time of hospitalization (23.75 ± 7.56 vs. 17.05 ± 6.20 ; $p<0.001$), higher rates of need for vasopressors (48% vs. 24.36%; $p = 0.004$), MV (29.33% vs. 7.69%; $p = 0.001$), and sedation (49.33% vs. 19.23%; $p<0.001$). The duration of MV, ICU stay, and hospital stay was significantly longer in this group (3.52 ± 8.78 vs. 0.29 ± 1.48 days; $p<0.001$, 10.53 ± 10.89 vs. 5.51 ± 3.03 days; $p = 0.002$, and 2.79 ± 4.94 vs. 1.09 ± 2.62 days; $p = 0.006$, respectively). Furthermore, the incidence of nosocomial infections was significantly higher in the readmission group than in the non-readmission group (52.70% vs. 5.13%; $p<0.001$). The mortality rate of these patients was 66.67%, whereas no mortality was observed in the group without readmission ($p<0.001$). The overall mortality rate of the cohort was 32.68%. Among comorbid diseases, dementia/AD ($p = 0.006$) and CVD history

($p = 0.035$) were more common in the readmission group (Table 1).

When laboratory findings were examined, serum albumin levels during both initial admission and transfer to the ward were lower in the readmitted group ($p<0.001$, $p<0.001$, respectively). Furthermore, the PNI was lower in the readmitted group at discharge ($p<0.001$). In contrast, the blood urea nitrogen (BUN) level at the time of admission ($p = 0.003$) and the fraction of inspired oxygen (FiO_2) demand ($p = 0.007$) were significantly higher in the readmitted group. The C-reactive protein level ($p = 0.006$) and pH value ($p = 0.028$) at the time of transfer to the ward were also higher in this group (Table 2).

Univariate and multivariate logistic regression analyses

According to Univariate Logistic Regression analysis, the variables found to be significantly associated with mortality were as follows: initial hospitalization GCS (OR=0.822, $p<0.001$), discharge before ICU GCS (OR=0.669, $p<0.001$), APACHE II score (OR=1.108, $p<0.001$), need for vasopressors (OR=2.433, $p=0.013$), duration of hospitalization (OR=1.132, $p=0.012$), duration of ICU stay (OR=1.048, $p=0.027$), presence of nosocomial infection (OR=4.919, $p<0.001$), low serum albumin levels (OR=0.198, $p<0.001$; OR=0.152, $p<0.001$ on transfer), high CRP (OR=1.004, $p=0.046$), history of dementia/Alzheimer's disease (OR=3.349, $p=0.016$) and history of CVD (OR=4.042, $p=0.011$). The number of MV days was significantly associated with mortality (OR = 1.051, $p = 0.085$) (Table 3).

Multivariate logistic regression analysis was performed using the backward Wald method. The initial model included the following clinically relevant variables: age, GCS score at ICU admission and discharge, APACHE II score, use of vasopressors, length of hospital and ICU stay, presence of nosocomial infection, serum albumin and CRP levels, and presence of dementia/Alzheimer's disease or CVD. After the stepwise elimination of non-significant variables, 3 variables independently affected mortality: APACHE II score (odds ratio [OR] = 1.095, 95% CI: 1.034-1.159, $p=0.002$), presence of nosocomial infection (OR = 2.746, 95% CI: 1.177-6.408, $p=0.019$) and albumin level measured during ICU admission (OR = 0.208, 95% CI: 0.095-0.459, $p<0.001$). These findings suggest that a high APACHE score and the presence of nosocomial infection increase mortality and that mortality increases as serum albumin levels decrease (Table 3).

The model's discriminative ability was assessed using an ROC curve, with an AUC value of 0.815. Model calibration was evaluated using the Hosmer-Lemeshow goodness-of-fit test, which yielded a p -value of 0.367, indicating an adequate fit. All variance inflation factor (VIF) values ranged from 1 to 4.3, suggesting that no significant multicollinearity was present among the independent variables.

Table 1. Clinical and demographic characteristics of patients with and without ICU readmission.

	Patients not requiring readmission (n=78) Mean±SD or n (%)	Patients requiring readmission (n=75) Mean±SD or n (%)	p-value*
Gender			0.595
Male	30 (38.46)	33 (44)	
Female	48 (61.54)	42 (56)	
Age	67.76 ± 15.13	75.25 ± 12.42	0.001
Site of ICU admission			0.065
In-Patient Ward	29 (37.18)	40 (53.33)	
Emergency Department	49 (62.82)	35 (46.67)	
Initial admission GCS score	14.56 ± 1.41	11.83 ± 4.14	<0.001
GCS before discharge	14.87 ± 0.54	13.17 ± 2.63	<0.001
APACHE II	17.05 ± 6.20	23.75 ± 7.56	<0.001
The Need for Vasopressors	19 (24.36)	36 (48)	0.004
Need for more than one vasopressor	1 (1.28)	2 (2.67)	0.615
Need for an MV	6 (7.69)	22 (29.33)	0.001
Number of MV days	0.29 ± 1.48	3.52 ± 8.78	<0.001
Need for NIMV	44 (56.41)	42 (56.00)	>0.999
Need for HFNC	12 (15.38)	13 (17.57)	0.886
Dialysis	3 (3.85)	4 (5.33)	0.716
Sedation	15 (19.23)	37 (49.33)	<0.001
Feeding			0.048
None	1 (1.28)	1 (1.33)	
Enterally	76 (97.44)	66 (88)	
Parenterally	0 (0)	5 (6.67)	
Enterally+Parenterally	1 (1.28)	3 (4)	
DM	19 (24.36)	29 (38.67)	0.083
CAD	22 (28.21)	18 (24)	0.683
COPD	38 (48.72)	29 (38.67)	0.276
HT	42 (53.85)	47 (62.67)	0.346
CHF	20 (25.64)	25 (33.33)	0.386
Rhythm Disorders	12 (15.38)	17 (22.67)	0.346
CRF	6 (7.69)	12 (16)	0.179
Extrapulmonary Malignant tumor	7 (8.97)	15 (20)	0.087
Lung Cancer	4 (5.13)	10 (13.33)	0.096
Dementia/Alzheimer Disease	4 (5.13)	15 (20)	0.006
CVD	4 (5.13)	12 (16)	0.035
One or more comorbidities	71 (91.03)	73 (97.33)	0.167
Days of hospitalization	1.09 ± 2.62	2.79 ± 4.94	0.006
Days in the ICU	5.51 ± 3.03	10.53 ± 10.89	0.002
hospital-acquired Infection	4 (5.13)	39 (52.70)	<0.001
Mortality	0 (0)	50 (66.67)	<0.001
Discharge to the ward			0.05
On working hours	61 (78.21)	47 (62.67)	
Out of Hours	17 (21.79)	27 (36.00)	
Not Known		1 (1.33)	

ICU: Intensive Care Unit, GCS: Glasgow Coma Scale, APACHE: Acute Physiology and Chronic Health Evaluation, COPD: Chronic Obstructive Pulmonary Disease, MV: Mechanical Ventilation, NIMV: Non-Invasive Mechanical Ventilation, HFNC: High-Flow Nasal Cannula, DM: Diabetes Mellitus, CAD: Coronary Artery Disease, HT: Hypertension, CHF: Congestive Heart Failure, CRF: Chronic Renal Failure, CVD: Cerebrovascular Disease. *Pearson's chi-squared test; Wilcoxon rank-sum test; Fisher's exact test.

■ DISCUSSION

In this study, patients who were readmitted to the ICU had higher mortality rates, and mortality was associated with certain comorbidities and prognostic, clinical, and laboratory findings.

In previous studies, the rate of re-admission to the ICU during the same hospitalization was 10% [2, 4]. In our study, this rate was as low as 5.56%, which may be due to the inclusion of only the pulmonary ICU. Not all patients requiring readmission were admitted to the pulmonary ICU; some were sent to

other ICUs or hospitals, potentially lowering the readmission rate.

Patient discharge from the ICU is based on the experience and subjective assessment of the ICU physician [9]. There has been an increasing interest in scoring systems in recent days. Although scoring systems developed for the evaluation of ICU readmission and mortality, such as the Stability and Workload Index for Transfer (SWIFT), Sequential Organ Failure Assessment (SOFA), and Therapeutic Intervention Scoring System (TISS-28), have moderate accuracy [10],

Table 2. Comparison of laboratory findings of patients with and without ICU readmission.

	Patients not requiring readmission (n=78) Mean±SD or n (%)	Patients requiring readmission (n=75) Mean±SD or n (%)	p-value*
At First Admission to the ICU			
CRP	86.04 ± 92.94	95.86 ± 93.55	0.256
PCT	4.54 ± 16.05	0.85 ± 2.36	0.633
pH	7.37 ± 0.09	7.38 ± 0.10	0.185
PO ₂	104.91 ± 33.12	100.66 ± 33.86	0.409
PCO ₂	46.59 ± 16.80	47.07 ± 17.16	0.745
FiO ₂	55.71 ± 20.11	64.40 ± 22.56	0.007
Lactate	1.68 ± 0.72	1.77 ± 1.17	0.474
WBC	13,881.92 ± 12,127.87	14,183.07 ± 8,980.77	0.354
Neutrophil	12,237.82 ± 11,673.91	11,501.07 ± 6,638.79	0.762
Platelet	267,512.82 ± 95,791.54	264,760.00 ± 103,713.07	0.865
Albumin	3.46 ± 0.56	3.08 ± 0.54	<0.001
BUN	27.67 ± 18.21	37.51 ± 22.89	0.003
Cr	1.25 ± 1.34	1.18 ± 1.00	0.911
PNI	39.66 ± 7.11	40.76 ± 31.37	0.012
PIV	3,216.23 ± 7,470.75	2,549.58 ± 4,886.23	0.621
Before discharge from the ICU			
CRP	39.56 ± 45.41	53.32 ± 43.72	0.006
PCT	0.58 ± 1.88	0.26 ± 0.33	0.096
pH	7.44 ± 0.04	7.45 ± 0.06	0.028
PO ₂	102.97 ± 29.38	104.59 ± 32.73	0.961
PCO ₂	46.58 ± 11.23	45.47 ± 10.56	0.584
FiO ₂	36.95 ± 8.52	36.73 ± 8.31	0.876
Lactate	1.32 ± 0.52	1.31 ± 0.53	0.635
WBC	9,642.44 ± 5,827.68	9,242.27 ± 4,765.75	0.969
Neutrophil	7,910.26 ± 5,844.76	6,974.40 ± 2,981.72	0.680
Platelet	255,435.90 ± 105,115.47	225,682.40 ± 117,752.56	0.063
Albumin	3.09 ± 0.47	2.75 ± 0.47	<0.001
BUN	23.06 ± 13.08	23.79 ± 14.86	0.964
Cr	0.91 ± 0.79	0.82 ± 0.70	0.169
PNI	36.27 ± 5.77	35.18 ± 18.70	<0.001
PIV	1,599.21 ± 3,057.26	1,014.54 ± 961.29	0.655

CRP: C-Reactive Protein, PCT: Procalcitonin, PO₂: Partial Oxygen Pressure, PCO₂: Partial Carbon dioxide Pressure, FiO₂: Fraction of Inspired Oxygen, WBC: White Blood Cell, BUN: Blood-Urea Nitrogen, Cr: Creatinine, PNI: Prognostic Nutritional Index, PIV: Pan-Immune Inflammation Value. *Wilcoxon rank sum test; Welch two-sample t-test.

Table 3. Univariate and multivariate analysis results on the risk factors for mortality.

Variables	Univariate		Multivariate*	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Age	1.042 (1.013-1.072)	0.005		
GCS score at admission	0.822 (0.739-0.914)	<0.001		
GCS before discharge	0.669 (0.553-0.809)	<0.001		
APACHE II	1.108 (1.055-1.165)	<0.001	1.095 (1.034-1.159)	0.002
The Need for Vasopressors	2.433 (1.210-4.893)	0.013		
Need for an MV	1.181 (0.500-2.789)	0.705		
Days of MV	1.051 (0.993-1.111)	0.085		
Days of hospitalization	1.132 (1.027-1.248)	0.012		
Days in the ICU	1.048 (1.005-1.093)	0.027		
Nosocomial Infections	4.919 (2.308-10.482)	<0.001	2.746 (1.177-6.408)	0.019
Albumin level (first admission)	0.198 (0.097-0.401)	<0.001	0.208 (0.095-0.459)	<0.001
Albumin level (before discharge)	0.152 (0.065-0.358)	<0.001		
CRP (first admission) level	1.004 (1.000-1.007)	0.046		
PNI (before discharge)	0.994 (0.964-1.024)	0.682		
Dementia/Alzheimer Disease	3.349 (1.252-8.960)	0.016		
CVD	4.042 (1.377-11.866)	0.011		

OR: Odds Ratio, SE: Standard Error, CI: Confidential Interval, GCS: Glasgow Coma Scale, APACHE: Acute Physiology and Chronic Health Evaluation, MV: Mechanical Ventilation, CRP: C-Reactive Protein, PNI: Prognostic Nutritional Index, CVD: Cerebrovascular Disease. Note: Model fit assessed via Hosmer-Lemeshow test (p = 0.367). AUC: 0.815. Multicollinearity was evaluated via VIF, all < 5. *Backward Wald method.

the clinician's opinion is still determinant in terms of easy applicability. In our study, we preferred the APACHE II score, which can be easily applied during hospitalization in all patients. The APACHE II score was higher in the group requiring readmission to the ICU, and mortality increased as the APACHE score increased. Our results support the literature [4, 11]. Therefore, we adjusted the APACHE II score for disease severity, which was included in the model as a potential confounder.

Patients requiring readmission were older, which is consistent with the literature [3, 4, 12]. Elderly patients tend to have higher frailty scores and are at greater risk for comorbidities than younger adults [12]. In our study, dementia/AD and CVD were particularly more common in the readmitted group. Low GCS both during admission and discharge to the ward was associated with neurological diseases. Another important question is whether these patients are better treated in palliative care than treated and/or readmitted to intensive care. Perhaps with improved palliative care centers, ICU admissions/readmissions can be reduced, together with costs, and patients who really need intensive care can be treated more effectively by reducing the length of stay in emergency departments. ICU hospitalizations account for 25%–40% of all health expenditures [13, 14].

Nutritional status is also more limited in elderly patients than in younger patients. Consistent with studies demonstrating that nutritional status is a prognostic marker for mortality [15], we observed lower albumin and PNI values in patients who were readmitted. However, low albumin levels may also be attributed to infection, as albumin is a negative acute-phase reactant, and nosocomial infections were more frequent in this group.

Vasopressor use increases in-hospital mortality, especially in elderly patients [15]. In our study, vasopressor use in ICUs was shown to increase mortality approximately 2.5-fold, in support of the literature.

Early discharge in ICUs is important to prevent intensive care infections and reduce costs. Prolonged ICU stay increases the risk of developing nosocomial infections [10]. In our study, both hospitalization duration and nosocomial infection rate were high in the readmission group. *Klebsiella pneumoniae* were the most common infectious agent, accounting for 25.6% of cases.

Respiratory failure and pneumonia are frequent causes of ICU readmission. Respiratory failure accounts for 18%–59% of all ICU readmissions [16]. This rate was higher in our unit because it is a pulmonary ICU. Because of hypoxic and hypercapnic respiratory failure, 70.7% of patients was readmitted to the ICU. We anticipate that such a high rate can be reduced with more participation of respiratory physiotherapists in the treatment.

Studies indicate that being discharged from ICUs during out-of-duty increases mortality [17]. Although we could not sta-

tistically prove this, the result supports this finding in our study. This has been attributed to a decrease in the number of staff and nurses working during off-duty hours. Another reason may be that waiting patients to be admitted to the emergency department may have led to early discharge from the ICU. Lack of nursing care and inappropriate treatment are among the preventable causes of ICU readmissions [18]. The key decision of the clinician is the optimal time for ward transfer.

These data reveal that ICU readmitted patients have worse clinical parameters and laboratory indicators, which are strongly associated with mortality. However, future multicenter randomized controlled studies with larger sample sizes are needed because this study is retrospective and includes a relatively small patient population.

Limitations

This study has certain limitations, the most notable being the relatively small sample size ($n = 153$). A larger sample size may be necessary in multivariable logistic regression models involving a large number of predictors to ensure model stability and statistical power. Although a power analysis was performed, the limited sample size may restrict the generalizability of the findings. Moreover, due to the lack of matching in the control group, differences may exist in certain confounding variables, which could have influenced the interpretation of the results.

This retrospective single-center study included only patients readmitted to the pulmonary ICU. Patients who were transferred to other ICUs were excluded, which may have led to an underestimation of the true ICU readmission rate. Therefore, the findings may reflect a limited perspective on overall readmission patterns.

CONCLUSION

Parameters such as the APACHE II score, serum albumin level, and the presence of nosocomial infection, which were found to be associated with mortality, may serve as valuable indicators for early risk stratification and the development of clinical management strategies in critically ill patients. Moreover, patients readmitted to the ICU had a higher risk of mortality, and readmission was closely associated with multiple clinical risk factors. Elderly patients and those with higher APACHE II and lower GCS scores and a history of dementia/AD may be at increased risk of readmission. Further multicenter studies should be performed to reveal risk factors for readmission and the benefit of close monitoring of high-risk patients, enhancing strategies for discharge planning, and improving transitional care, which may reduce readmissions and improve outcomes in critically ill patients more precisely.

Ethics Committee Approval: This study was approved by the Scientific Research Ethics Committee of the Ministry of Health, Istanbul Health Sciences University,

Umraniye Training and Research Hospital (01.08.2024; B.10.1.TKH.4.34.H.GP.0.01/234). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Informed Consent: The study was retrospective, informed consent was not obtained.

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Author Contributions: AÇ: Materials, Data Collection and/or Processing, Analysis and/or Interpretation, Writing; ŞB: Conception, Design, Supervision, Critical Review.

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■ REFERENCES

1. Chan KS, Tan CK, Fang CS, Tsai CL, Hou CC. et al. Readmission to the intensive care unit: an indicator that reflects the potential risks of morbidity and mortality of surgical patients in the intensive care unit. *Surg Today*. 2009;39(4):295-9. doi: [10.1007/s00595-008-3876-6](https://doi.org/10.1007/s00595-008-3876-6).
2. Ponzoni CR, Corrêa TD, Filho RR, Serpa Neto A, Assunção MS, Pardini A, Schettino GP. Readmission to the intensive care unit: incidence, risk factors, resource use, and outcomes. *Ann Am Thorac Soc*. 2017;14(8):1312-1319. doi: [10.1513/AnnalsATS.201611-851OC](https://doi.org/10.1513/AnnalsATS.201611-851OC).
3. Kramer AA, Higgins TL, Zimmerman JE. Intensive care unit readmissions in US hospitals: patient characteristics, risk factors, and outcomes. *Crit Care Med*. 2012 Jan;40(1):3-10. doi: [10.1097/CCM.0b013e31822d751e](https://doi.org/10.1097/CCM.0b013e31822d751e).
4. Wong EG, Parker AM, Leung DG, Brigham EP, Arbaje AI. Association of severity of illness and intensive care unit readmission: a systematic review. *Heart Lung*. 2016;45(1):3-9. e2. doi: [10.1016/j.hrtlng.2015.10.040](https://doi.org/10.1016/j.hrtlng.2015.10.040).
5. Kareliusson F, De Geer L, Tibblin AO. Risk prediction of ICU readmission in a mixed surgical and medical population. *J Intensive Care*. 2015;3(1):30. doi: [10.1186/s40560-015-0096-1](https://doi.org/10.1186/s40560-015-0096-1).
6. Özkarakas H, Öztürk MC. Readmissions to intensive care from palliative care units: risk factors, incidence, and Outcome. *İstanbul Med J*. 2024;25(1):72-76. doi: [10.4274/imj.galenos.2024.59852](https://doi.org/10.4274/imj.galenos.2024.59852).
7. Markazi-Moghaddam N, Fathi M, Ramezankhani A. Risk prediction models for intensive care unit readmission: a systematic review of methodology and applicability. *Aust Crit Care*. 2020;33(4):367-374. doi: [10.1016/j.aucc.2019.05.005](https://doi.org/10.1016/j.aucc.2019.05.005).
8. Rojas JC, Lyons PG, Jiang T, Kilaru M, McCauley L. et al. Accuracy of clinicians' ability to predict the need for intensive care unit readmission. *Ann Am Thorac Soc*. 2020;17(7):847-853. doi: [10.1513/AnnalsATS.201911-828OC](https://doi.org/10.1513/AnnalsATS.201911-828OC).
9. Mahmoodpoor A, Sanaie S, Saghaleini SH, Ostadi Z, Hosseini MS. et al. Prognostic value of National Early Warning Score and Modified Early Warning Score on intensive care unit readmission and mortality: a prospective observational study. *Front Med (Lausanne)*. 2022;9:938005. doi: [10.3389/fmed.2022.938005](https://doi.org/10.3389/fmed.2022.938005).
10. Rosa RG, Roehrig C, Oliveira RPD, Maccari JG, Antônio ACP. et al. Comparison of unplanned intensive care unit readmission scores: a prospective cohort study. *PLoS One*. 2015;10(11):e0143127. doi: [10.1371/journal.pone.0143127](https://doi.org/10.1371/journal.pone.0143127).
11. Lin W-T, Chen W-L, Chao C-M, Lai C-C. The outcomes and prognostic factors of the patients with unplanned intensive care unit readmissions. *Medicine (Baltimore)*. 2018;97(26):e11124. doi: [10.1097/MD.00000000000011124](https://doi.org/10.1097/MD.00000000000011124).
12. Woldhek AL, Rijkenberg S, Bosman RJ, Van Der Voort PH. Readmission of ICU patients: A quality indicator? *J Crit Care*. 2017;38:328-334. doi: [10.1016/j.jcrc.2016.12.001](https://doi.org/10.1016/j.jcrc.2016.12.001).
13. Ruppert MM, Loftus TJ, Small C, Li H, Ozrazgat-Baslanti T. et al. Predictive modeling for readmission to intensive care: a systematic review. *Crit Care Explor*. 2023;5(1):e0848. doi: [10.1097/CCE.0000000000000848](https://doi.org/10.1097/CCE.0000000000000848).
14. Kaben A, Corrêa F, Reinhart K, Settmacher U, Gummert J. et al. Readmission to a surgical intensive care unit: incidence, outcome, and risk factors. *Crit Care*. 2008;12(5):R123. doi: [10.1186/cc7023](https://doi.org/10.1186/cc7023).
15. Lee S-I, Koh Y, Huh JW, Hong S-B, Lim C-M. Factors and outcomes of intensive care unit readmission in elderly patients. *Gerontology*. 2022;68(3):280-288. doi: [10.1159/000516297](https://doi.org/10.1159/000516297).
16. Mcneill H, Khairat S. Impact of intensive care unit readmissions on patient outcomes and the evaluation of the national early warning score to prevent readmissions: literature review. *JMIR Perioper Med*. 2020;3(1):e13782. doi: [10.2196/13782](https://doi.org/10.2196/13782).
17. Vollam S, Dutton S, Lamb S, Petrinic T, Young JD, Watkinson P. Out-of-hours discharge from intensive care, in-hospital mortality and intensive care readmission rates: a systematic review and meta-analysis. *Intensive Care Med*. 2018;44(7):1115-1129. doi: [10.1007/s00134-018-5245-2](https://doi.org/10.1007/s00134-018-5245-2).
18. Al-Jaghbeer MJ, Tekwani SS, Gunn SR, Kahn JM. Incidence and etiology of potentially preventable ICU readmissions. *Crit Care Med*. 2016;44(9):1704-1709. doi: [10.1097/CCM.0000000000001746](https://doi.org/10.1097/CCM.0000000000001746).



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Should videolaryngoscopy enter routine use? Unanticipated difficult airway: A five-year experience in a tertiary care hospital

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■ MAIN POINTS

- VL significantly improved glottic visualization in patients with UDA, reducing the median Cormack-Lehane grade from 3 (DL) to 1 (VL) ($p<0.001$).
- VL achieved a 97% success rate in patients who could not be intubated with DL, suggesting its strong effectiveness as more than just a rescue tool.
- Compared to DL, VL was associated with significantly lower rates of esophageal intubation (1.5% vs. 9.8%) and oropharyngeal injury (2.3% vs. 11.9%) ($p<0.001$), demonstrating its potential to reduce airway-related complications.
- The study supports the consideration of VL as a first-line technique for airway management, especially in patients with normal airway assessment but unexpected intubation difficulty.
- Routine use of VL may improve patient safety and overall intubation success in UDA cases, warranting its broader integration into airway management protocols.

■ ABSTRACT

Aim: An unanticipated difficult airway (UDA) can be very challenging for anesthesiologists in airway management and, if not managed appropriately, may lead to increased morbidity and even mortality in patients. Because patients with normal physical examination findings may also have a difficult airway, meticulous preparations are essential for every patient. Our study investigated the five-year outcomes of a tertiary care hospital in patients with UDA.

Materials and Methods: We retrospectively reviewed the records of 143 patients with UDA who underwent surgery under general anesthesia between January 2020 and March 2025. We evaluated their airway management: preoperative physical examination findings, demographics, comorbidities, mask ventilation, laryngoscopic visualization, and tracheal intubation success.

Results: We found that videolaryngoscopy (VL) improved glottic visualization compared to direct laryngoscopy (DL) in patients with UDA ($p<0.001$). VL reduced the risk of complications and accidental esophageal intubation compared to DL ($p<0.001$, $p<0.001$, respectively). We also found that 129 out of 133 patients (97%) who could not be intubated with DL were successfully intubated endotracheally using VL.

Conclusion: VL improves glottic visualization, reduces the risk of complications, and increases the rate of successful intubation compared to DL in patients with UDA.

Keywords: Anesthesia, Airway management, Difficult airway, Intubation, Laryngoscopy, Videolaryngoscopy

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■ INTRODUCTION

Airway management is a cornerstone of safe anesthesia practice and is usually achieved by endotracheal intubation. A difficult airway is defined as challenges in mask ventilation, laryngoscopic visualization, tracheal intubation, or the need for an emergency surgical airway [1,2]. Among these, UDA is particularly critical, as it occurs in patients without identifiable predictors during preoperative evaluation [3,4]. If not promptly recognized and managed, UDA may result in hypoxemia, airway trauma, and even mortality [5].

Preoperative predictors such as Mallampati classification, thyromental distance, interincisor gap, neck mobility, and upper lip bite test are commonly used but have limited sensitivity and specificity [6–8]. Thus, patients with apparently normal examinations may still present with unexpected difficulties, highlighting the need for structured airway management strategies and access to alternative devices [9,10].

When intubation fails, maintaining oxygenation becomes the priority. International guidelines recommend adjuncts such as VL, supraglottic devices, or flexible intubation scopes be-

fore invasive techniques or awakening the patient [3,11]. VL provides an indirect view of the glottis, improves visualization in difficult cases, and reduces esophageal intubation and airway trauma [12–14]. However, limitations such as cost, reduced effectiveness in restricted mouth opening, and tube delivery challenges remain [15].

VL has been incorporated into national and international difficult airway guidelines [4,16], but its role as a first-line tool rather than a rescue device is still debated, especially in UDA cases.

The present study analyzed a five-year experience in a tertiary care center, focusing on the role of VL compared to DL in UDA. Specifically, we investigated its effects on glottic visualization, intubation success, and airway-related complications.

■ MATERIALS AND METHODS

Ethical approval for this study was received from Tokat Gaziosmanpaşa University Clinical Research Ethics Committee (25-MOBAEK-124) on April 8, 2025, and the retrospective observational study was registered at ClinicalTrials.gov (NCT06972394) on May 13, 2025. This study was designed as a retrospective observational study and was conducted in accordance with the STROBE guidelines for observational studies and the principles of the Declaration of Helsinki.

Study population and sampling

Data on patients with UDA who underwent elective surgery under general anesthesia between January 2020 and March 2025 were obtained by reviewing hospital automation systems, patient records, and difficult airway forms. All eligible cases during this period were included consecutively (non-probability consecutive sampling). We excluded patients with missing data, a history of difficult airway, or known difficult airway findings. No randomization or blinding was performed due to the retrospective nature of the study.

Outcomes

The primary outcome was the success rate of tracheal intubation with VL compared with DL. Secondary outcomes included glottic visualization (Cormack–Lehane grade), complications such as esophageal intubation and oropharyngeal injury, and intubation difficulty scores (IDS).

Airway management protocol

In our clinic, preoperative airway evaluation includes assessment of Mallampati classification, thyromental distance, sternomental distance, interincisor gap, neck circumference, upper lip bite test, neck mobility, dentition, hoarseness, exertional dyspnea, and history of previous difficult airway. Patients without predictors of a difficult airway underwent routine intubation using DL in the sniffing position.

Management of patients with UDA was carried out according to a specific protocol. The C-MAC® D-Blade (Karl Storz,

Tuttlingen, Germany) VL with a hyper-angled blade was used as a rescue technique in patients who could not be intubated with DL, provided that mask ventilation and oxygenation were maintained. If tracheal intubation could not be achieved with VL and the patient was awakened, awake flexible intubation scope (FIS) was planned when general anesthesia was required.

Mask ventilation difficulty was classified as Class I–IV. IDS was calculated by evaluating seven variables, and a score greater than five indicated difficult intubation [16].

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA), licensed through Tokat Gaziosmanpaşa University. The normality of distribution for quantitative variables was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Normally distributed continuous variables were summarized as mean $\pm$ standard deviation, while non-normally distributed variables were expressed as median (minimum–maximum). Categorical variables were presented as numbers and percentages.

Comparisons of categorical variables such as intubation success, esophageal intubation, and oropharyngeal injury between groups were performed using Fisher’s Exact Test or Pearson’s Chi-Square Test, with continuity correction where appropriate. Paired comparisons of Cormack–Lehane scores between DL and VL in the same patients were conducted using the non-parametric Wilcoxon Signed-Rank Test. Correlation analyses were performed using Spearman’s rho (r). However, due to the study design, in which all patients initially underwent DL and only those with failed DL were subsequently managed with VL, the two groups were not statistically independent. Therefore, while group comparisons were performed as described, the results should be interpreted with caution given this dependency.

All tests were two-tailed, and a p-value <0.05 was considered statistically significant. Exact p-values are reported in the results and tables.

■ RESULTS

We evaluated 355 patients for the study. 201 patients had a difficult airway findings or history, while 11 had missing data. The study included 143 patients. Figure 1 shows the study’s flow chart. The median (min–max) patient age was 53 years (18–75 years), with 57% being male. Fifty-eight patients were smokers, 46 had hypertension, 32 had diabetes mellitus, and 22 had chronic lung disease. The patients’ demographic data and descriptive characteristics are in Table 1.

In our study, DL was initially attempted in all 143 patients with UDA. Among these, successful intubation was achieved in 10 cases using DL. The remaining 133 patients, in whom DL failed, were subsequently intubated using VL. VL was

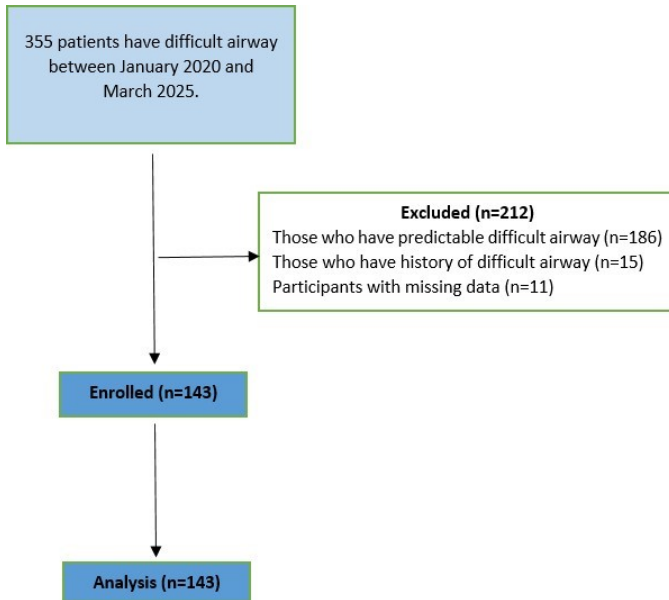


Figure 1. Flow diagram of the study.

Table 1. Baseline characteristics and clinical outcomes of the study population.

Age (years)	53 (18 – 75)
Sex (Female / Male), n (%)	61 / 82 (43 / 57)
BMI (kg/m ²)	27.77 (19.1 – 29.78)
ASA (I / II / III), n (%)	42 / 84 / 17 (29 / 59 / 12)
Hypertension, n (%)	46 (32)
Diabetes, n (%)	32 (22)
Ischemic heart disease, n (%)	17 (12)
Chronic lung disease, n (%)	22 (15)
Thyroid disease, n (%)	7 (5)
Cerebral vascular disease, n (%)	3 (2)
Neoplasm, n (%)	13 (9)
Rheumatic disease, n (%)	20 (14)
Smoking, n (%)	58 (41)
Chronic kidney disease, n (%)	3 (2)
Mallampati classification (I / II), n (%)	54 / 89 (38 / 62)
Neck circumference (normal), n (%)	143 (100)
Upper lip bite test (1 / 2), n (%)	103 / 40 (72 / 28)
Thyromental distance (normal), n (%)	143 (100)
Neck mobility ($\geq 80^\circ$), n (%)	143 (100)
Interincisor gap ≥ 3.5 cm, n (%)	143 (100)
IDS	7 (6 – 9)
Difficult mask ventilation (I / II / III), n (%)	85 / 52 / 6 (59 / 37 / 4)

BMI: body mass index; ASA: American Society of Anesthesiologists; IDS: intubation difficulty scale; quantitative data are given as median (minimum – maximum).

successful in 129 of these cases, while 4 patients could not be intubated with either method and were awakened. These four patients were awakened and prepared for elective awake intubation with preservation of spontaneous breathing, and successful tracheal intubation was achieved using the FIS.

The overall intubation success rate was 7% with DL and 97% with VL, and this difference was statistically significant ($p < 0.001$) (Table 2). However, it is important to emphasize that the groups were not independent. Since VL was used only in patients where DL had already failed, this indicates a selection of more difficult cases in the VL group. Therefore,

the remarkably high success rate of VL, suggests not only a statistically but also a clinically significant advantage of VL.

The median (min–max) Cormack-Lehane grade value was three (2–4) in DL and one (1–3) in VL (Table 2). VL significantly decreased the Cormack-Lehane grade compared to DL ($p < 0.001$), (Table 2). This supports the advantage of VL in providing better visualization of the glottic structures.

Seventeen of 143 patients (11.9%) in DL and three of 133 patients (2.3%) in VL had oropharyngeal injury during attempted endotracheal intubation (Table 2). VL significantly reduced the risk of complications compared to DL ($p < 0.001$) (Table 2). We observed esophageal intubation during endotracheal intubation attempts in 14 of 143 patients (9.8%) with DL and two of 133 patients (1.5%) with VL (Table 2). VL significantly decreased the risk of accidental esophageal intubation compared to DL ($p < 0.001$) (Table 2).

The patients had a median (min–max) IDS value of seven (6–9). All patients had an IDS score above five, indicating difficult intubation. In the current study, the incidence of difficult airway in elective surgeries was found to be 1.97%, while the incidence of UDA was found to be 0.79%. In addition, mask ventilation was easy in 85 patients (59.4%), moderately difficult in 52 (36.4%), and highly difficult in six (4.2%), while none had impossible mask ventilation.

The relationship between the parameters in airway management was as follows (Table 3): We found a moderate positive correlation between IDS score and mask ventilation difficulty, oropharyngeal injury in DL, and esophageal intubation in DL; a weak positive correlation between IDS score and CL score in DL, CL score in VL, esophageal intubation in VL, and oropharyngeal injury in VL; and a weak negative correlation between IDS score and intubation success in VL and intubation success in DL.

There was a weak positive correlation between mask ventilation difficulty and CL score in DL, oropharyngeal injury in DL, oropharyngeal injury in VL, esophageal intubation in VL, and esophageal intubation in DL; and a weak negative correlation between mask ventilation difficulty and intubation success in VL.

There was a good negative correlation between CL score and intubation success in DL; a weak positive correlation between CL score in DL and oropharyngeal injury in VL, oropharyngeal injury in DL, esophageal intubation in VL, and esophageal intubation in DL; and a weak negative correlation between CL score in DL and intubation success in VL. We found a moderate positive correlation between CL score in VL and esophageal intubation in DL; a weak positive correlation between CL score in VL and oropharyngeal injury in VL, oropharyngeal injury in DL and esophageal intubation in VL; and a moderate negative correlation between CL score in VL and intubation success in VL.

There was a strong positive correlation between oropharyngeal injury in VL and esophageal intubation in VL; a moder-

Table 2. Comparison of the effects of different laryngoscopes on airway management.

	Direct Laryngoscopy	Videolaryngoscopy	p
CL score, median (min-max)	3 (2 - 4)	1 (1 - 3)	<0.001 ^{a*}
Successful intubation, n (%)	10 (7)	129 (97)	<0.001 ^{b*}
Esophageal intubation, n (%)	14 (9.8)	2 (1.5)	<0.001 ^{c*}
Oropharyngeal injury, n (%)	17 (11.9)	3 (2.3)	<0.001 ^{c*}

^a: Wilcoxon Signed Ranks; ^b: Fisher's Exact test; ^c: Pearson Chi-Square test; *: statistically significant; CL: Cormack-Lehane classification; Quantitative data are given as median (minimum - maximum).

Table 3. Relationship between difficulties in airway management, tracheal intubation success and complications.

	IDS	Difficult mask ventilation	CL score with DL	CL score with VL	Oropharyngeal injury with VL	Oropharyngeal injury with DL	Esophageal intubation with VL	Esophageal intubation with DL	Successful intubation with DL	Successful intubation with VL
IDS	rho p									
Difficult mask ventilation	rho p	0.516* <0.001								
CL score with DL	rho p	0.251* 0.003	0.231* 0.005							
CL score with VL	rho p	0.368* <0.001	-0.089 0.308	0.073 0.401						
Oropharyngeal injury with VL	rho p	0.241* 0.005	0.246* 0.004	0.328* <0.001	0.360* <0.001					
Oropharyngeal injury with DL	rho p	0.552* <0.001	0.380* <0.001	0.234* 0.005	0.271* 0.002	0.417* <0.001				
Esophageal intubation with VL	rho p	0.206* 0.022	0.212* 0.006	0.208* 0.016	0.290* 0.001	0.813* <0.001	0.323* <0.001			
Esophageal intubation with DL	rho p	0.455* <0.001	0.248* 0.003	0.229* 0.006	0.504* <0.001	0.443* <0.001	0.606* <0.001	0.360* <0.001		
Successful intubation with DL	rho p	-0.217* 0.011	-0.072 0.390	-0.616* <0.001			-0.101 0.231		-0.090 0.283	
Successful intubation with VL	rho p	-0.266* 0.002	-0.208* 0.013	-0.297* 0.001	-0.403* <0.001	-0.566* <0.001	-0.460* <0.001	-0.340* <0.001	-0.513* <0.001	

rho: Spearman's rho correlation coefficient; *: p < 0.05. (IDS: intubation difficulty scale; DL: direct laryngoscopy; VL: videolaryngoscopy; CL: Cormack-Lehane classification).

ate positive correlation between oropharyngeal injury in VL and oropharyngeal injury in DL and esophageal intubation in DL; and a moderate negative correlation between oropharyngeal injury in VL and intubation success in VL. We found a good positive correlation between oropharyngeal injury in DL and esophageal intubation in DL, a low positive correlation between oropharyngeal injury in DL and esophageal intubation in VL, and a moderate negative correlation between oropharyngeal injury in DL and intubation success in VL. There was a weak positive correlation between esophageal intubation in VL and esophageal intubation in DL; and a weak negative correlation between esophageal intubation in VL and intubation success in VL. We also found a moder-

ate negative correlation between esophageal intubation in DL and intubation success in VL.

■ DISCUSSION

UDA presents a significant challenge for practitioners and is one of the leading causes of increased morbidity associated with anesthesia. Especially when not properly managed, it can lead to complications that may result in patient death. Our study showed that VL improves glottic visualization and increases endotracheal intubation success compared to DL in UDA. We also found that VL reduced the risk of oropharyngeal injury and esophageal intubation compared to DL. A preoperative comprehensive airway evaluation, along with

diagnostic imaging when necessary, assessment of the patient's physiological status (including apnea tolerance, aspiration risk, and hemodynamic status), and a review of the patient's previous airway management history provide valuable information for identifying potential difficult airways. However, a difficult airway can also be seen in patients with normal screening tests. The use of VL should be considered in patients with difficult intubation on DL, as it facilitates endotracheal intubation and increases the success rate [17].

VL has been included in airway management guidelines as its benefits have become more recognized over time, and its prevalence and familiarity with its use have increased [4,13]. Many studies have suggested including VL in routine clinical practice in airway management rather than used in failed intubation with DL [13,18,19]. VL is also an alternative to flexible bronchoscopy in awake intubation of appropriate patients [20]. Studies have reported that it provides advantages in increasing intubation success, reducing the rate of difficult laryngoscopy and improving glottic visualization, reducing airway trauma and the risk of hypoxia, and defining esophageal intubation better [17,21]. Nevertheless, the high cost, lack of familiarity with its use, lens fogging, and secretions and blood obstructing the camera's view limit VL use [9]. In addition, VL is not recommended if the mouth opening is limited (<2.5 cm), the cervical spine is fixed in flexion, and there is a tumor in or near the airway accompanied by stridor [14]. Consistent with the literature, the present study showed that VL improved glottic visualization, reduced the risk of complications, and increased the rate of successful intubation compared to DL in patients with UDA.

Patients with difficult or failed tracheal intubation are more likely to have difficult mask ventilation, and patients with difficult mask ventilation are more likely to have difficult or failed tracheal intubation [22,23]. Similarly, patients with failed supraglottic airway ventilation have been shown to have a higher incidence of difficult face mask ventilation [24]. This is referred to as "compound failure of airway management" as tracheal intubation and mask ventilation share common predictors of difficulty [22]. In our study, 40.6% of the patients experienced difficulty in mask ventilation (36.4% moderately difficult, 4.2% highly difficult), which is higher than the literature [2,3]. This may be because all patients in the study had difficult intubation [22,23].

Awake intubation with preservation of the patient's spontaneous breathing should be considered as a potentially safer option when difficulty is anticipated in two or more of the airway management stages. Intubation with awake FIS performed by experienced people has been reported to have a high success and low complication rate under appropriate conditions and in appropriate patients [25]. We found four patients with failed tracheal intubation with both DL and VL were successfully intubated using FIS, with no complications observed.

The risk of airway-related complications (esophageal intubation, aspiration, and oropharyngeal damage) is higher in

patients with difficult intubation compared to others and is reported to vary between 4.1% and 28% [26]. In the present study, the rate of airway-related complications was 23.1% (esophageal intubation 9.1%, oropharyngeal injury 14%), consistent with the literature. Of these complications, 84.8% occurred in DL and 15.2% in VL.

In the study conducted by Norskov et al., the incidence of UDA was reported as 1.87% [27]. Alemdar et al. found that 24.9% of adult patients with difficult airways had UDA [28]. Furthermore, Endlich et al. reported that in 10% of difficult or failed intubation cases, no predictive factor could be identified [29]. In the present study, the incidence of difficult airway in elective surgeries was found to be 1.97%, while the incidence of UDA was 0.79%.

Limitations

Our study has some limitations worth mentioning. Firstly, it was retrospective. However, a prospective study is unlikely to be performed in these patients as difficult airways cannot be predicted. Secondly, the airway evaluation was based solely on the patient's physical examination findings and anesthesia history. Advanced assessment methods such as ultrasound, awake nasal endoscopy, or oral videolaryngoscopy, which could provide more detailed information regarding airway anatomy and management, were not utilized in this study. These imaging and evaluation techniques may offer potential benefits in reducing the incidence of UDA. However, their routine use in many clinical settings is significantly limited due to being time-consuming and cost-prohibitive. Thirdly, this study focused solely on patients undergoing elective surgery. The incidence of difficult airways and associated complications may be higher in emergency surgical cases. Another limitation is that all patients were from a single center, and only the C-MAC® D-Blade VL with a hyper-angled barrel was used as a rescue technique for those with failed intubation with DL. Further studies are needed to investigate the effects of VLs with different characteristics on airway management in a larger patient population from various regions, especially in UDA.

CONCLUSION

The present study showed that VL improved glottic visualization, reduced the risk of complications such as oropharyngeal injury and esophageal intubation, and increased the rate of successful endotracheal intubation compared to DL in patients with UDA. Therefore, our results support VL as a routine technique rather than as a rescue method for failed DL. It could significantly reduce the risk of adverse events in patients with UDA. However, we believe it should be confirmed in more patient groups and with different VL types.

Ethics Committee Approval: This retrospective study involving human participants was in accordance with the ethical standards of the institutional and national research committee

and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Approval was granted by the Ethics Committee of Tokat Gaziosmanpaşa University (April 8, 2025 / 25-MOBAEK-124).

Informed Consent: Due to the retrospective design of the study, the requirement for informed consent to participate was waived.

Data availability: The data used to support the findings of this study can be obtained from the corresponding author on request.

Peer-review: Externally peer-reviewed.

Conflict of Interest: The authors have no relevant financial or non-financial interests to disclose.

Author Contributions: Conception: A.G; Design: A.G, H.T, T.K, S.K; Supervision: A.G, V.K, H.T, T.K, S.K; Materials: A.G, M.G.B, T.K; Data Collection and/or Processing: A.G, A.T.S, H.T; Analysis and/or Interpretation: A.G, A.T.S, M.G.B, T.K, S.K; Literature Review: A.G, V.K; Writing: A.G, A.T.S, M.G.B, V.K, H.T, T.K, S.K; Critical Review: A.T.S, M.G.B, V.K, H.T, T.K, S.K.

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■ REFERENCES

1. Yemam D, Melese E, Ashebir Z. Comparison of modified mallampati classification with Cormack and Lehane grading in predicting difficult laryngoscopy among elective surgical patients who took general anesthesia in Werabie comprehensive specialized hospital - Cross sectional study. Ethiopia, 2021. *Ann Med Surg (Lond)*. 2022;79:103912. doi: [10.1016/j.amsu.2022.103912](https://doi.org/10.1016/j.amsu.2022.103912).
2. Nørskov AK, Wetterslev J, Rosenstock C, Afshari A, Astrup G, Jakobsen J, et al. Prediction of difficult mask ventilation using a systematic assessment of risk factors vs. existing practice—a cluster randomised clinical trial in 94,006 patients. *Anaesthesia*. 2017;72(3):296-308. doi: [10.1111/anae.13701](https://doi.org/10.1111/anae.13701).
3. Law JA, Duggan LV, Asselin M, Baker P, Crosby E, Downey A, et al. Canadian Airway Focus Group updated consensus-based recommendations for management of the difficult airway: part 1. Difficult airway management encountered in an unconscious patient. *Can J Anaesth*. 2021;68(9):1373-404. doi: [10.1007/s12630-021-02007-0](https://doi.org/10.1007/s12630-021-02007-0).
4. Law JA, Duggan LV, Asselin M, Baker P, Crosby E, Downey A, et al. Canadian Airway Focus Group updated consensus-based recommendations for management of the difficult airway: part 2. Planning and implementing safe management of the patient with an anticipated difficult airway. *Can J Anaesth*. 2021;68(9):1405-36. doi: [10.1007/s12630-021-02008-z](https://doi.org/10.1007/s12630-021-02008-z).
5. Rosenblatt W, Ianus AI, Sukhupragarn W, Fickenscher A, Sasaki C. Preoperative endoscopic airway examination (PEAE) provides superior airway information and may reduce the use of unnecessary awake intubation. *Anesth Analg*. 2011;112(3):602-7. doi: [10.1213/ANE.0b013e3181fd1c](https://doi.org/10.1213/ANE.0b013e3181fd1c).
6. Sakles JC, Pacheco GS, Kovacs G, Mosier JM. The difficult airway refocused. *Br J Anaesth*. 2020;125(1):e18-e21. doi: [10.1016/j.bja.2020.04.008](https://doi.org/10.1016/j.bja.2020.04.008).
7. Kornas RL, Owyang CG, Sakles JC, Foley LJ, Mosier JM, Committee obotSfAMsSP. Evaluation and Management of the Physiologically Difficult Airway: Consensus Recommendations From Society for Airway Management. *Anesth Analg*. 2021;132(2):395-405. doi: [10.1213/ANE.0000000000005233](https://doi.org/10.1213/ANE.0000000000005233).
8. Marchis IF, Negrut MF, Blebea CM, Crihan M, Alexa AL, Breazu CM. Trends in Preoperative Airway Assessment. *Diagnostics (Basel)*. 2024;14(6):610. doi: [10.3390/diagnostics14060610](https://doi.org/10.3390/diagnostics14060610).
9. Martins MP, Ortenzi AV, Perin D, Quintas G, Malito ML, Carvalho VH. Recommendations from the Brazilian Society of Anesthesiology (SBA) for difficult airway management in adults. *Braz J Anesthesiol*. 2024;74:744477. doi: [10.1016/j.bjane.2023.12.001](https://doi.org/10.1016/j.bjane.2023.12.001).
10. Tsai Y-CM, Russotto V, Parotto M. Predicting the Difficult Airway: How Useful Are Preoperative Airway Tests? *Curr Anesthesiol Rep*. 2022;12(3):398-406. doi: [10.1007/s40140-022-00525-1](https://doi.org/10.1007/s40140-022-00525-1).
11. Yuan J, Ye H, Tan X, Zhang H, Sun J. Determinants of difficult laryngoscopy based on upper airway indicators: a prospective observational study. *BMC Anesthesiol*. 2024;24(1):157. doi: [10.1186/s12871-024-02543-4](https://doi.org/10.1186/s12871-024-02543-4).
12. Gómez-Ríos MÁ, Sastre JA, Onrubia-Fuertes X, López T, Abad-Gurumeta A, Casans-Francés R, et al. Spanish Society of Anesthesiology, Reanimation and Pain Therapy (SEDAR), Spanish Society of Emergency and Emergency Medicine (SEMES) and Spanish Society of Otolaryngology, Head and Neck Surgery (SEORL-CCC) Guideline for difficult airway management. Part II. *Rev Esp Anesthesiol Reanim (Engl Ed)*. 2024;71(3):207-47. doi: [10.1016/j.redare.2024.02.002](https://doi.org/10.1016/j.redare.2024.02.002).
13. Saul SA, Ward PA, McNarry AF. Airway management: The current role of videolaryngoscopy. *J Pers Med*. 2023;13(9):1327. doi: [10.3390/jpm13091327](https://doi.org/10.3390/jpm13091327).
14. Apfelbaum JL, Hagberg CA, Connis RT, Abdelmalak BB, Agarkar M, Dutton RP, et al. 2022 American Society of Anesthesiologists Practice Guidelines for Management of the Difficult Airway. *Anesthesiology*. 2022;136(1):31-81. doi: [10.1097/ALN.0000000000004002](https://doi.org/10.1097/ALN.0000000000004002).
15. Zaouter C, Calderon J, Hemmerling T. Videolaryngoscopy as a new standard of care. *Br J Anaesth*. 2015;114(2):181-183. doi: [10.1093/bja/aeu266](https://doi.org/10.1093/bja/aeu266).
16. Prakash S, Mullick P, Singh R. Evaluation of thyromental height as a predictor of difficult laryngoscopy and difficult intubation: a cross-sectional observational study. *Braz J Anesthesiol*. 2022;72(06):742-8. doi: [10.1016/j.bjane.2021.07.001](https://doi.org/10.1016/j.bjane.2021.07.001).
17. Lewis SR, Butler AR, Parker J, Cook TM, Smith AF. Videolaryngoscopy versus direct laryngoscopy for adult patients requiring tracheal intubation. *Cochrane Database Syst Rev*. 2016(11). doi: [10.1002/14651858.CD011136.pub2](https://doi.org/10.1002/14651858.CD011136.pub2).
18. Cook TM, Aziz MF. Has the time really come for universal videolaryngoscopy? *Br J Anaesth*. 2022;129(4):474-477. doi: [10.1016/j.bja.2022.07.038](https://doi.org/10.1016/j.bja.2022.07.038).
19. Chrimes N, Higgs A, Hagberg C, Baker P, Cooper R, Greif R, et al. Preventing unrecognised oesophageal intubation: a consensus guideline from the Project for Universal Management of Airways and international airway societies. *Anaesthesia*. 2022;77(12):1395-415. doi: [10.1111/anae.15817](https://doi.org/10.1111/anae.15817).
20. Wilson WM, Smith AF. The emerging role of awake videolaryngoscopy in airway management. *Anaesthesia*. 2018;73(9):1058-61. doi: [10.1111/anae.14324](https://doi.org/10.1111/anae.14324).
21. Hansel J, Rogers AM, Lewis SR, Cook TM, Smith AF. Videolaryngoscopy versus direct laryngoscopy for adults undergoing tracheal intubation. *Cochrane Database Syst Rev*. 2022(4). doi: [10.1002/14651858.CD011136.pub3](https://doi.org/10.1002/14651858.CD011136.pub3).
22. Cook T, MacDougall-Davis S. Complications and failure of airway management. *Br J Anaesth*. 2012;109(suppl_1):i68-i85. doi: [10.1093/bja/aes393](https://doi.org/10.1093/bja/aes393).
23. Nørskov AK, Rosenstock C, Wetterslev J, Astrup G, Afshari A, Lundstrøm L. Diagnostic accuracy of anaesthesiologists' prediction of difficult airway management in daily clinical practice: a cohort study of 188 064 patients registered in the Danish Anaesthesia Database. *Anaesthesia*. 2015;70(3):272-81. doi: [10.1111/anae.12955](https://doi.org/10.1111/anae.12955).
24. Vannucci A, Rossi IT, Prifti K, Kallogjeri D, Rangrass G, De-Cresce D, et al. Modifiable and nonmodifiable factors associated with perioperative failure of extraglottic airway devices. *Anesth Analg*. 2018;126(6):1959-67. doi: [10.1213/ANE.00000000000002659](https://doi.org/10.1213/ANE.00000000000002659).

25. El-Boghdady K, Onwochei D, Cuddihy J, Ahmad I. A prospective cohort study of awake fiberoptic intubation practice at a tertiary centre. *Anaesthesia*. 2017;72(6):694-703. doi: [10.1111/anae.13844](https://doi.org/10.1111/anae.13844).
26. Martin LD, Mhyre JM, Shanks AM, Tremper KK, Kheterpal S. 3,423 Emergency Tracheal Intubations at a University Hospital: Airway Outcomes and Complications. *Anesthesiology*. 2011;114:42-48. doi: [10.1097/ALN.0b013e318201c415](https://doi.org/10.1097/ALN.0b013e318201c415).
27. Nørskov AK, Rosenstock CV, Wetterslev J, Lundstrøm LH. Incidence of unanticipated difficult airway using an objective airway score versus a standard clinical airway assessment: the DIFFICAIR trial – trial protocol for a cluster randomized clinical trial. *Trials*. 2013;14(1):347. doi: [10.1186/1745-6215-14-347](https://doi.org/10.1186/1745-6215-14-347).
28. Alemdar D, Akesen S, Bilgin H. Retrospective Investigation of Difficult Airway Cases Encountered in Bursa Uludağ University Medical Faculty Operating Room. *Turk J Anaesthesiol Reanim*. 2023;51(2):121-7. doi: [10.5152/TJAR.2023.22213](https://doi.org/10.5152/TJAR.2023.22213).
29. Endlich Y, Lee J, Culwick MD. Difficult and failed intubation in the first 4000 incidents reported on webAIRS. *Anesth Crit Care*. 2020;48(6):477-87. doi: [10.1177/0310057X2095765](https://doi.org/10.1177/0310057X2095765).



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A resveratrol-loaded scaffold enhances tendon healing: Histological and biomechanical analysis in a rat Achilles tendon repair model

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■ MAIN POINTS

- This experimental study evaluates the effects of a novel bioabsorbable scaffold (Dermalix®), composed of collagen, laminin, and resveratrol-loaded microparticles, on tendon healing in a rat Achilles tendon model.
- Histological analysis demonstrated reduced fibroblast proliferation in the Dermalix® group compared to controls.
- Biomechanical testing showed significantly higher maximum load and stiffness values in the Dermalix® group at both 3 and 6 weeks post-operatively.
- Dermalix® appears to reduce peritendinous adhesions and may offer a promising adjunct for tendon repair surgeries by promoting organized healing.

■ ABSTRACT

Aim: This study aims to evaluate the effects of Dermalix® (Dx), a resveratrol-loaded, bioabsorbable scaffold composed of collagen, laminin, and hyaluronic acid, on tendon healing and peritendinous adhesion formation following primary Achilles tendon repair in a rat model.

Materials and Methods: A total of 28 female Wistar Albino rats underwent bilateral Achilles tendon injury and repair using the modified Kessler technique. The right legs were treated with local Dx application over the repair site, while the left legs served as untreated controls. Animals were randomly assigned to one of four groups (n = 7 per group). The groups were defined by the evaluation time point (3 or 6 weeks) and the type of analysis (histological or biomechanical). Assessments included macroscopic adhesion scoring, histological quantification (counts of fibroblasts, fibrocytes, and vessels), and biomechanical testing (maximum load to failure and elongation at rupture). Statistical comparisons were made using t-test, Mann-Whitney U, chi-square, ANOVA, or the Friedman test, as appropriate for the data.

Results: Dx application significantly reduced severity of adhesion at both 3 and 6 weeks (p<0.01). Histological analyses revealed significantly lower fibroblast and fibrocyte counts with more organised collagen alignment in Dx-treated groups (p<0.05). Biomechanically, rupture force was significantly higher in Dx groups at both time points (p<0.01 and p=0.029), while no significant difference was found in elongation distance. The scaffold was resorbed by week 3, without inducing any foreign body reaction or systemic side effects.

Conclusion: The local application of Dx enhanced tendon healing significantly and reduced peritendinous adhesions without compromising biomechanical strength. Its bioabsorbable composition and local antioxidant properties make Dx a promising supportive local treatment option in tendon repair surgery. This is particularly valid in cases with high adhesion risk.

Keywords: Resveratrol, Bioabsorbable scaffold, Tendon healing, Peritendinous adhesion, Achilles tendon, Dermalix®

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■ INTRODUCTION

Tendon injuries are frequently encountered in clinical practice and may result in prolonged functional limitations, even following technically successful repairs [1]. Multiple therapeutic approaches have been investigated to enhance tendon regeneration. These approaches include biological agents

such as cytokines, growth factors, and stem cells, as well as physical treatments like hyperbaric oxygen therapy. In addition, various biomaterials have been studied, including platelet concentrates, hyaluronic acid (HA), stem cell-derived microvesicles, and zinc oxide nanoparticles [2–4]. Although many adjuvant therapies have shown promise in both preclin-

ical and clinical studies, none have improved tendon healing consistently enough to justify routine adoption, highlighting the urgent need for new research to reduce the risk of long-term impairments [5,6].

Phytoalexins, found in many vegetables, especially grapevines, are protective substances synthesised by plants against ultraviolet radiation, bacterial and fungal infections [7]. Resveratrol (RSV) is a phytoalexin and has many benefits that stems from its potent antioxidant properties. Dermalix® (Abdi İbrahim İlaç San. ve Tic. A.Ş., Turkey) (Dx) is a patented bioabsorbable scaffold composed of collagen, laminin, and RSV-loaded microparticles. Initially developed for treatment of diabetic wounds. Its supportive role in the treatment of chronic wounds, regenerative potential and antioxidant properties suggest Dx may also enhance tendon repair [8–10].

This study aimed to investigate the effects of Dx, used as an adjuvant therapy, on the quality of tendon healing, healing rate, and peritendinous adhesion formation following primary tendon repair.

MATERIALS AND METHODS

Study design

This prospective, controlled, experimental animal study was approved by the Local Ethics Committee for Animal Experiments (Approval Date-No: 21.03.2023, 2023-05/08). All procedures were conducted in compliance with the Helsinki Declaration and international guidelines for the care and use of laboratory animals, including the ARRIVE guidelines. Measures were taken to minimise animal suffering, pain, and distress throughout the study. Housing, feeding, and care of the experimental animals were conducted by the principles outlined in the “Guide for the Care and Use of Laboratory Animals”. Appropriate anaesthesia and analgesia protocols were applied to reduce postoperative pain and ensure animal welfare.

The sample size was determined based on previous studies evaluating biomechanical outcomes in tendon healing models [11]. To detect a 25% difference in mean rupture force between groups, with a standard deviation of 20%, an α -error of 0.05, and 80% power, the minimum required sample size was calculated as 7 tendons per group (effect size: 1.45). The analysis was performed using G*Power version 3.1.9.7 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany).

Surgical model and experimental groups

Twenty-eight female Wistar Albino rats (250–300 g; 3–4 months old) were acquired from Bursa Uludağ University's Experimental Animal Research Unit (BUÜ - DENHAB). A convenience sampling method was used.

In each rat, a bilateral surgical procedure was performed on the Achilles tendons. Under anesthesia, a midline skin incision was made to expose the Achilles and plantaris tendons. The Achilles tendon was transected 0.3–0.5 cm proximal to

its calcaneal insertion. To create a critical-size defect that impairs spontaneous recovery, a 3 mm segment of the tendon was excised. The plantaris tendon was left intact to serve as an internal splint. The severed Achilles tendon was then repaired using a modified Kessler technique with 5/0 non-absorbable suture.

A paired-limb design was used for treatment comparison. In each animal, the right leg was designated the treatment group, where Dx was applied to the repair line before skin closure (Figure 1). The left leg served as the control group and received no adjuvant therapy.



Figure 1. Intraoperative Photograph Demonstrating Dermalix® Application Over the Surgically Exposed Rat Achilles Tendon. Intraoperative view of the rat hind limb showing the exposed and surgically transected Achilles tendon following repair. Dermalix® scaffold has been applied over the repair site to facilitate healing and minimize peritendinous adhesions.

Following surgery, animals were housed at 20–22°C and 50–55% humidity with ad libitum access to food and water. The 28 rats were randomly allocated into four cohorts (n=7 per cohort) based on the planned endpoint analysis:

- Histology Week 3 (Hist3): Sacrificed at 3 weeks for histological evaluation.
- Biomechanics Week 3 (Bmc3): Sacrificed at 3 weeks for biomechanical testing.
- Histology Week 6 (Hist6): Sacrificed at 6 weeks for histological evaluation.
- Biomechanics Week 6 (Bmc6): Sacrificed at 6 weeks for biomechanical testing.

Outcome assessments

To minimize observer bias, all histological and biomechanical assessments were performed by investigators blinded to the group allocations.

- **Macroscopic Adhesion Scoring:** Adhesion formation was graded using a semiquantitative scale: grade 1 (none), grade 2 (filmy), grade 3 (mild), grade 4 (moderate, 35–60% of area), and grade 5 (severe, >60% of area) [12].
- **Histological Analysis:** Tendon samples were fixed in 10% formaldehyde, embedded in paraffin, and sectioned at 5 μm . Sections were stained with haematoxylin and eosin (H&E) to assess tissue morphology and Masson's trichrome (MT) to evaluate collagen organization. Quantitative analysis included counts of fibroblasts, fibrocytes, and blood vessels oriented parallel to collagen fibers.
- **Biomechanical Testing:** Tendons were mounted in a tensile testing machine. After measuring the tendon diameter with a digital caliper, a tensile load was applied at a constant displacement rate of 10 mm/min until failure. The maximum rupture force and stiffness were recorded (Figure 2).

Primary endpoints

The primary endpoints were the histological quality of tendon healing (collagen fiber alignment, cellular density) and biomechanical tensile strength (maximum rupture force).

Statistical analysis

Statistical analysis was performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean $\pm$ standard deviation (SD), and categorical variables as frequencies and percentages. Normality was assessed with the Shapiro-Wilk test. Between-group comparisons (Control vs. Dermalix) were made using the independent samples t-test or Mann-Whitney U test, depending on data distribution. The Chi-square test or Fisher's exact test was used for categorical variables. Intragroup comparisons over time were analyzed using repeated measures ANOVA or the Friedman test, with Bonferroni correction applied for post-hoc analyses. Spearman's correlation analysis was used to assess relationships between histological and biomechanical variables. $P < 0.05$ was considered statistically significant. P values were reported with two digits, or three digits if starting with zeros (e.g., $P = 0.002$). Values close to .05 were expressed to three decimals (e.g., $P = 0.053$); values < 0.001 were reported as $P < 0.001$.



Figure 2. Mounting of the Achilles Tendon Specimen for Biomechanical Testing. Achilles tendon sample clamped between the opposing jaws of the tensile testing machine prior to biomechanical load-to-failure testing.

RESULTS

Macroscopic findings

Macroscopic evaluation revealed that the treatment significantly reduced adhesion formation. At both the 3- and 6-week time points, the control groups (CG) exhibited moderate to severe adhesions, with no instances of mild or no adhesions. In contrast, the majority of rats in the treatment groups (DxG) developed either no or only mild adhesions. This difference between the control and treatment groups was statistically significant ($p < 0.01$, Table 2).

Histological evaluation

Histological analysis with H&E and MT staining demonstrated superior collagen organization in the treatment groups (Figure 3). Tendons from the control groups were characterized by a disorganized, dense collagen pattern. Conversely, tendons from the treatment groups showed a more regular and aligned collagen fiber structure, indicative of more mature healing.

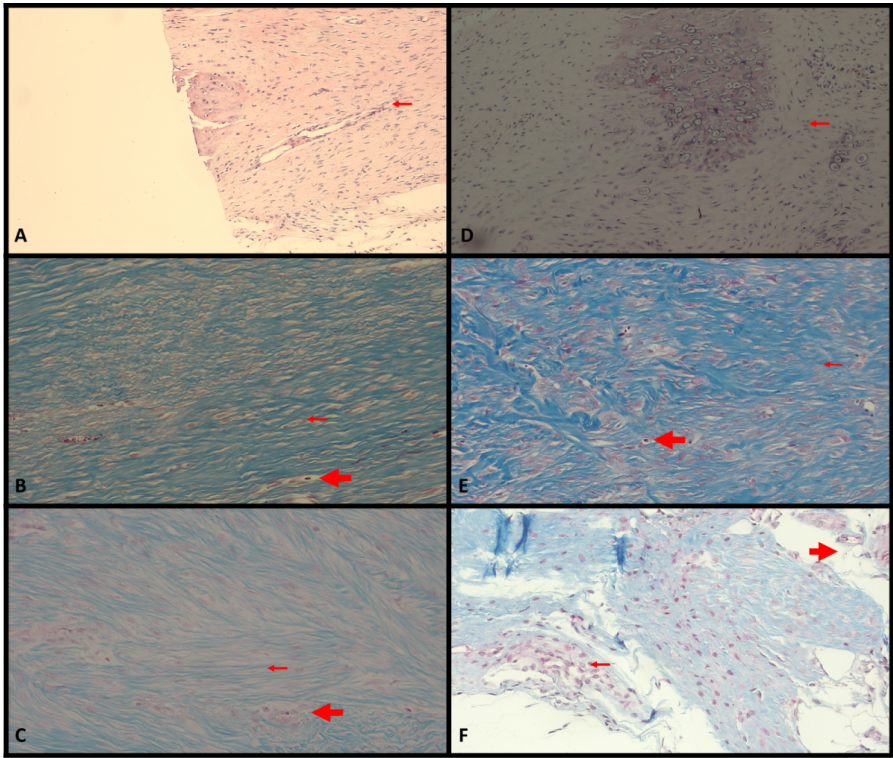


Figure 3. Representative Histological Images of Tendon Healing at 3rd and 6th Weeks in Dermalix and Control Groups. Dermalix Group: (a) 3rd Week Hematoxylin and Eosin Staining, (b) 3rd Week Masson's Trichrome Staining, (c) 6th Week Masson's Trichrome Staining. Control Group: (d) 3rd Week Hematoxylin and Eosin Staining, (e) 3rd Week Masson's Trichrome Staining, (f) 6th Week Masson's Trichrome Staining. Thin red arrows indicate fibroblasts; thick red arrows demonstrate areas of neovascularization.

Table 1. Grouping of samples and procedures to be applied.

		Hist3 n=7 Rat (=14 tendons)		Bmc3 n=7 Rat (=14 tendons)		Hist6 n=7 Rat (=14 tendons)		Bmc6 n=7 Rat (=14 tendons)	
		Hist3-DxG	Hist3-CG	Bmc3-DxG	Bmc3-CG	Hist6-DxG	Hist6-CG	Bmc6-DxG	Bmc6-CG
Number of tendons included in the study		7	7	7	7	7	7	7	7
Initial Procedures	Rupture model created	✓	✓	✓	✓	✓	✓	✓	✓
	Repair	✓	✓	✓	✓	✓	✓	✓	✓
	Dx Application	✓		✓		✓		✓	
Week 3 Procedures	Histological Evaluation (Healing and Adhesion)	✓	✓			✓	✓		
	Biomechanical Evaluation			✓	✓			✓	✓
Week 6 Procedures	Histological Evaluation (Healing and Adhesion)	✓	✓			✓	✓		
	Biomechanical Evaluation			✓	✓			✓	✓

Dx: Dermalix®, Hist3-DxG: Dermalix Histology Group Week 3, Hist3-CG: Control Histology Group Week 3, Bmc3-DxG: Dermalix Biomechanics Group Week 3, Bmc3-CG: Control Biomechanics Group Week 3, Hist6-DxG: Dermalix Histology Group Week 6, Hist6-CG: Control Histology Group Week 6, Bmc6-DxG: Dermalix Biomechanics Group Week 6, Bmc6-CG: Control Biomechanics Group Week 6.

Quantitative analysis showed that the treatment significantly reduced cellular density at the repair site (Table 3). At week 3, the mean number of fibroblasts ($p=0.032$) and fibrocytes ($p<0.001$) was significantly lower in the DxG compared to the CG. This reduction in cellularity persisted at week 6 for both fibroblasts ($p=0.002$) and fibrocytes ($p<0.001$). No significant differences in neovascularization were observed between groups at either time point ($p>0.05$).

Biomechanical testing

Biomechanical testing demonstrated that the treatment significantly improved the tensile strength of the repaired tendons. At week 3, the maximum load to failure was significantly higher in the DxG than in the CG ($p<0.0001$). This enhanced strength was maintained through week 6, with the DxG again showing a significantly greater maximum load compared to the CG ($p=0.029$). Although the elongation

Table 2. Macroscopic adhesion results of all groups.

	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Hist3-CG	0	0	1	3	3
Hist3-DxG	1	3	3	0	0
Hist6-CG	0	0	0	3	4
Hist6-DxG	3	3	1	0	0

Hist3-DxG: Dermalix Histology Group Week 3, Hist3-CG: Control Histology Group Week 3, Hist6-DxG: Dermalix Histology Group Week 6, Hist6-CG: Control Histology Group Week 6.

Table 3. Mean number of fibroblasts, fibrocytes and neovascularisation of all groups.

	Hist3-CG	Hist3-DxG	P	Hist6-CG	Hist6-DxG	P
Fibroblast (n, SD)	218.51±65.92	188.37±47.82	0.032	141.05±22.32	100.62±35.89	0.002
Fibrocyte (n, SD)	32.11±26.58	13.22±13.20	<0.001	136.91±31.75	71.88±7.05	<0.001
Neovascularisation (n, SD)	5.08±2.01	6.82±4.30	0.655	4.60±2.42	3.45±1.72	0.870

Hist3-DxG: Dermalix Histology Group Week 3, Hist3-CG: Control Histology Group Week 3, Hist6-DxG: Dermalix Histology Group Week 6, Hist6-CG: Control Histology Group Week 6, SD: Standard Deviation.

Table 4. Mean force and distance values of the rupture moment.

	Bmc3-CG	Bmct3-DxG	P	Bmct6-CG	Bmc6-DxG	P
Force (Newton, SD)	9.11±0.91	15.40±3.35	<0.001	36.45±13.76	42.94±10.88	0.029
Distance (mm, SD)	3.74±0.76	3.86±0.78	0.998	4.05±1.72	5.49±1.67	0.204

Bmc3-DxG: Dermalix Biomechanics Group Week 3, Bmc3-CG: Control Biomechanics Group Week 3, Bmc6-DxG: Dermalix Biomechanics Group Week 6, Bmc6-CG: Control Biomechanics Group Week 6.

at rupture was greater in the treatment groups at both time points, these differences did not reach statistical significance (Table 4).

■ DISCUSSION

This study investigated the efficacy of a bioabsorbable scaffold loaded with resveratrol (RSV), laminin, and hyaluronic acid (HA), referred to as Dermalix® (Dx), as an adjunctive treatment for primary tendon repair in a rat model. Our findings demonstrate that the local application of this scaffold significantly enhanced the biomechanical strength and histological quality of tendon healing while markedly reducing the formation of peritendinous adhesions.

Initially developed in 2014 by Eroğlu et al. as part of a TUBITAK project, the formulation consists of RSV-loaded hyaluronic acid/Dipalmitoyl phosphatidylcholine microparticles embedded in a 3D collagen-laminin matrix. The patented product (PCT/TR2014/000251) was demonstrated to accelerate wound healing in diabetic rats and was subsequently commercialised in 2018. A prospective clinical study conducted by Çetinkalp et al. confirmed that the local application of Dx reduced oxidative stress in the wound area and was absorbed entirely within 72 hours [8–10].

The positive outcomes align with a growing body of evidence supporting the use of plant-derived bioactive agents in tissue regeneration. Studies show the anti-inflammatory, cardioprotective, neuroprotective, antidiabetic, antitumor, antiviral and anticancer effects of RSV, the main active ingredient in

Dx [13,14]. Busch et al. linked the effect of RSV on tenocytes to the upregulation of SIRT-1 genes and the reduction of inflammatory mediators, such as Interleukin-1 Beta. They suggested that it could be used for tendonitis treatments in the future [15]. An animal study reported the positive effects of systemic RSV use on tendon healing [16]. There are also studies showing that it is effective and has no side effects, whether used systemically or locally [17,18]. Recent studies have further supported the therapeutic potential of RSV in tendon healing, especially in metabolically compromised conditions. In a 2021 experimental study, RSV treatment was shown to have protective effects on tendons in an animal model of obesity and insulin resistance by reducing the activity of matrix metalloproteinases (MMP-2 and MMP-9) and maintaining the protein content of tendon tissue. These findings emphasize the role of RSV in preventing catabolism and stability of tendons which is very important in tendon healing [19].

As highlighted in a recent review, RSV's potent antioxidant and anti-inflammatory properties are known to promote cellular function and tissue repair. Our results support the hypothesis that local delivery of RSV via a bioabsorbable matrix can effectively modulate the healing environment [20]. These data align with the findings of the present study, which showed that an RSV-loaded scaffold significantly enhanced tendon healing quality and biomechanical strength, while also reducing adhesion formation. This supports the hypothesis that RSV, when delivered locally through a bioabsorbable matrix, may represent a promising adjunctive strategy in tendon repair.

Another molecule included in Dx is laminin, a protein associated with the basal lamina that was shown to be positive in murine tendon epitenon by Taylor et al [21]. As a key component of the extracellular matrix, laminin plays a multifaceted role in fibrosis and tendon repair, mediating tissue integrity, cell adhesion, migration, and differentiation. It has been shown that the extracellular matrix is not only structural but also actively directs cellular behaviour during fibrosis development, and proteins such as laminin are decisive in this process [22]. In tendon repair, laminin plays a critical role in achieving biological success criteria such as the orientation of scleraxis-expressing cells, axial collagen alignment, and organization of the tendon-bone junction [23]. It has been reported that laminin-211 provides mechanical stability to the muscle-tendon junction through its association with dystroglycan and integrin $\alpha\beta1$ receptors in cell-matrix interactions, and its deficiency leads to functional loss [24]. Both fibronectin and laminin staining are found in the granulation tissue of healing tendons, indicating that these outer cells are involved in the early healing process [25].

Furthermore, the inclusion of laminin and HA likely contributed to the observed benefits. Laminin, a key extracellular matrix (ECM) protein, is critical for cell adhesion, migration, and the organization of the tendon-bone junction. Similarly, HA is well-established as an anti-adhesion agent that improves tissue gliding without impairing healing. The multifaceted composition of the Dx scaffold appears to create a synergistic effect, enhancing multiple aspects of tendon repair.

Throughout the study, the scaffold was observed to be completely bioabsorbed by the 3-week time point, with no macroscopic remnants or signs of a foreign body reaction. This confirms its suitability as a biodegradable local delivery vehicle that does not require subsequent removal. No signs of systemic side effects or foreign body reactions were observed in any of the treated animals. Despite the lack of pharmacokinetic data, the absence of visible remnants supports the scaffold's complete degradation and localised effect profile.

In a study by Zeytin et al. tendon healing was evaluated histologically and biomechanically on day 14 following systemic RSV administration in diabetic rats. Although the maximum tensile strength was higher in the RSV group, the difference did not reach statistical significance. Histologically, better healing patterns were observed in the RSV-treated group [16]. However, the study relied on semi-quantitative microscopic assessments, and to our knowledge, no previous research has performed quantitative histological evaluation. A key finding of our study was the significant reduction in cellularity (fibroblasts and fibrocytes) in the Dx-treated groups at both 3 and 6 weeks. While early fibroblast proliferation is essential for healing, excessive or prolonged cellular activity can lead to disorganized fibrosis and adhesion formation. Our quantitative analysis, which contrasts with the semi-quantitative methods of previous studies, suggests that the Dx scaffold promotes a more regulated and efficient healing process. The

lower cell density, combined with the observed improvement in collagen fiber alignment, indicates a favorable modulation of the inflammatory and proliferative phases of healing, which typically conclude by the third week in rats.

There is evidence demonstrating that Dx accelerates wound healing by mechanically supporting the growth of healing cells, as shown in a previous study. [9]. The biomechanical data further support this interpretation. Tendons treated with Dx demonstrated significantly higher rupture force at both 3 and 6 weeks, suggesting an accelerated transition to the remodeling phase and an earlier restoration of tensile strength. This enhanced mechanical integrity may be due to both the improved collagen organization and the initial structural support provided by the scaffold itself. Interestingly, neovascularization did not differ between the groups, suggesting the primary mechanism of action for Dx involves modulating the cellular and ECM environment rather than directly promoting angiogenesis. Further studies involving specific angiogenic markers could offer more detailed insights into this aspect.

Since Dx also contains hyaluronic acid as part of its bioactive scaffold, our findings are in line with previous studies that reported favourable outcomes with HA-based anti-adhesion agents and high-molecular-weight HA injections [26,27]. These agents were shown to improve range of motion and reduce peritendinous adhesions without impairing tendon healing, which supports the potential of Dx as a multifunctional adjuvant in tendon repair.

Limitations

This study has several limitations. A paired-limb design was used (treatment vs. control in the same animal), which carries a potential risk of systemic crossover effects from the bioactive agents. However, the localized application and significant between-limb differences suggest this effect was minimal. The sample size was relatively small, which may limit the generalizability of our findings. The assessment of collagen alignment was qualitative; future studies would benefit from quantitative image analysis to confirm these observations. Finally, the 6-week follow-up period only covers the early-to-intermediate stages of healing, and long-term studies are needed to assess the final maturation of the tendon and the persistence of the anti-adhesion effect.

This is the first study to use this specific scaffold for subcutaneous tendon repair, and further research is needed to compare its efficacy against other anti-adhesion barriers.

CONCLUSION

This preliminary animal study demonstrated that the local application of an RSV-loaded, bioabsorbable scaffold (Dermalix®) significantly reduced peritendinous adhesion formation and enhanced tendon healing, without compromising biomechanical strength. These results indicate that Dx may be a promising adjunct in primary tendon repairs, especially

when the risk of adhesion is high. Further research with larger sample sizes, long-term follow-up, and comparisons with other biomaterials is needed to confirm its clinical potential.

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Ethics Committee Approval: All animal procedures were approved by the Bursa Uludağ University Local Ethics Committee for Animal Experiments (Approval number: 2023-03/12) and were conducted under institutional guidelines and national regulations for the care and use of laboratory animals (European Directive 2010/63/EU).

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■ REFERENCES

- Thomopoulos S, Parks WC, Rifkin DB, Derwin KA. Mechanisms of tendon injury and repair. *J Orthop Res*. 2015;33(6):832-9. doi: [10.1002/JOR.22806](https://doi.org/10.1002/JOR.22806).
- Gökkaya A, Görgü M, Karanfil E, Acuner B. Does the use of Dermojet affect the concentration of platelet-rich plasma? An in vitro experimental investigation. *Dermatol Ther*. 2021;34(4):e14996. doi: [10.1111/DTH.14996](https://doi.org/10.1111/DTH.14996).
- Dincel YM, Adanir O, Arikan Y, Caglar AK, Dogru SC, Arslan YZ. Effects of High-Dose Vitamin C and Hyaluronic Acid on Tendon Healing. *Acta Orthop Bras*. 2018;26(2):82-85. doi: [10.1590/1413-785220182602182353](https://doi.org/10.1590/1413-785220182602182353).
- Adi Praja Semara Putra K. Addition of Hyperbaric Oxygen Therapy vs Standard Therapy in SSNHL. *KESANS*. 2022;1(4):391-395. doi: [10.54543/kesans.v1i4.49](https://doi.org/10.54543/kesans.v1i4.49).
- Ye YJ, Zhou YQ, Jing ZY, Liu YY, Yin DC. Electrospun Heparin-Loaded Core-Shell Nanofiber Sutures for Achilles Tendon Regeneration In Vivo. *Macromol Biosci*. 2018;18(7):e1800041. doi: [10.1002/mabi.201800041](https://doi.org/10.1002/mabi.201800041).
- Bosch G, Moleman M, Barneveld A, van Weeren PR, van Schie HT. The effect of platelet-rich plasma on the neovascularization of surgically created equine superficial digital flexor tendon lesions. *Scand J Med Sci Sports*. 2011;21(4):554-61. doi: [10.1111/j.1600-0838.2009.01070.x](https://doi.org/10.1111/j.1600-0838.2009.01070.x).
- Aribal-Kocatürk P, Kavas GO, Büyükkagıncı DI. Pretreatment effect of resveratrol on streptozotocin-induced diabetes in rats. *Biol Trace Elem Res*. 2007;118(3):244-9. doi: [10.1007/s12011-007-0031-y](https://doi.org/10.1007/s12011-007-0031-y).
- Eroglu İ, Gökçe EH, Tsapis N, Tanrıverdi ST, Gökçe G, et al. Evaluation of characteristics and in vitro antioxidant properties of RSV loaded hyaluronic acid-DPPC microparticles as a wound healing system. *Colloids Surf B Biointerfaces*. 2015;126:50-7. doi: [10.1016/j.col-surf.2014.12.006](https://doi.org/10.1016/j.col-surf.2014.12.006).
- Gokce EH, Tuncay Tanrıverdi S, Eroglu I, Tsapis N, Gokce G, et al. Wound healing effects of collagen-laminin dermal matrix impregnated with resveratrol loaded hyaluronic acid-DPPC microparticles in diabetic rats. *Eur J Pharm Biopharm*. 2017;119:17-27. doi: [10.1016/j.ejpb.2017.04.027](https://doi.org/10.1016/j.ejpb.2017.04.027).
- Özer Ö. Comparative Evaluation of Clinical Efficacy and Safety of Collagen Laminin-Based Dermal Matrix Combined With Resveratrol Microparticles (Dermalix) and Standard Wound Care for Diabetic Foot Ulcers. *Int J Low Extrem Wounds*. 2021;20(3):217-226. doi: [10.1177/1534734620907773](https://doi.org/10.1177/1534734620907773).
- Çolak M, Uzun B, Öztuna V. Tendon Hasarı Modelleri. In: Öztuna F. V., editor. *Ortopedi ve Travmatolojide Deneyisel Hayvan Modelleri*. 1st ed. Ankara: *TOTBİD*. pp. 173-193.
- Türküzü T, Güven N, Altındağ F, Tokyay A, Gökalg MA, Ismailov U, Uyumaz MA, Akkol S. Can pirfenidone prevent tendon adhesions? An experimental study in rats. *Jt Dis Relat Surg*. 2023;34(2):396-404. doi: [10.52312/JDRS.2023.1012](https://doi.org/10.52312/JDRS.2023.1012).
- Das S, Das DK. Anti-inflammatory responses of resveratrol. *Inflamm Allergy Drug Targets*. 2007;6(3):168-73. doi: [10.2174/187152807781696464](https://doi.org/10.2174/187152807781696464).
- Leon-Galicia I, Diaz-Chavez J, Garcia-Villa E, Uribe-Figueroa L, Hidalgo-Miranda A, et al. Resveratrol induces downregulation of DNA repair genes in MCF-7 human breast cancer cells. *Eur J Cancer Prev*. 2013;22(1):11-20. doi: [10.1097/CEJ.0b013e328353edcb](https://doi.org/10.1097/CEJ.0b013e328353edcb).
- Busch F, Mobasher A, Shayan P, Stahlmann R, Shakibaei M. Sirt-1 is required for the inhibition of apoptosis and inflammatory responses in human tenocytes. *J Biol Chem*. 2012;287(31):25770-81. doi: [10.1074/jbc.M112.355420](https://doi.org/10.1074/jbc.M112.355420).
- Zeytin K, Ciloğlu NS, Ateş F, Vardar Aker F, Ercan F. The effects of resveratrol on tendon healing of diabetic rats. *Acta Orthop Traumatol Turc*. 2014;48(3):355-62. doi: [10.3944/AOTT.2014.13.0096](https://doi.org/10.3944/AOTT.2014.13.0096).
- Shimazu Y, Shibuya E, Takehana S, Sekiguchi K, Oshima K, et al. Local administration of resveratrol inhibits excitability of nociceptive wide-dynamic range neurons in rat trigeminal spinal nucleus caudalis. *Brain Res Bull*. 2016;124:262-8. doi: [10.1016/j.brainresbull.2016.06.001](https://doi.org/10.1016/j.brainresbull.2016.06.001).
- Takehana S, Sekiguchi K, Inoue M, Kubota Y, Ito Y, et al. Systemic administration of resveratrol suppress the nociceptive neuronal activity of spinal trigeminal nucleus caudalis in rats. *Brain Res Bull*. 2016;120:117-22. doi: [10.1016/J.BRAINRESBULL.2015.11.011](https://doi.org/10.1016/J.BRAINRESBULL.2015.11.011).
- Da Ré Silva TM., Ferreira da Cruz SH., Majolli Andre D., Pires Marques P., Prado de Oliveira L, et al. Resveratrol Treatment Protects Tendons from Obesity and Insulin Resistance Effects. *Muscle, Ligaments and Tendons Journal*. 2021;11(4):628-634. doi: [10.32098/mltj.04.2021.03](https://doi.org/10.32098/mltj.04.2021.03).
- Li Y, Wang W, Xu W. Mechanisms and new advances in the efficacy of plant active ingredients in tendon-bone healing. *J Orthop Surg Res*. 2025;20(1):106. doi: [10.1186/s13018-025-05483-y](https://doi.org/10.1186/s13018-025-05483-y).
- Taylor SH, Al-Youha S, Van Agtmael T, Lu Y, Wong J, McGrouther DA, Kadler KE. Tendon is covered by a basement membrane epithelium that is required for cell retention and the prevention of adhesion formation. *PLoS One*. 2011;6(1):e16337. doi: [10.1371/journal.pone.0016337](https://doi.org/10.1371/journal.pone.0016337).
- Klingberg F, Hinz B, White ES. The myofibroblast matrix: implications for tissue repair and fibrosis. *J Pathol*. 2013;229(2):298-309. doi: [10.1002/PATH.4104](https://doi.org/10.1002/PATH.4104).
- Breidenbach AP, Gilday SD, Lalley AL, Dymont NA, Gooch C, et al. Functional tissue engineering of tendon: Establishing biological success criteria for improving tendon repair. *J Biomech*. 2014;47(9):1941-8. doi: [10.1016/J.JBIOMECH.2013.10.023](https://doi.org/10.1016/J.JBIOMECH.2013.10.023).
- Gawlik KI, Durbéej M. Skeletal muscle laminin and MDC1A: pathogenesis and treatment strategies. *Skelet Muscle*. 2011;1(1):9. doi: [10.1186/2044-5040-1-9](https://doi.org/10.1186/2044-5040-1-9).
- Howell K, Chien C, Bell R, Laudier D, Tufa SF, et al. Novel Model of Tendon Regeneration Reveals Distinct Cell Mechanisms Underlying Regenerative and Fibrotic Tendon Healing. *Sci Rep*. 2017;7:45238. doi: [10.1038/srep45238](https://doi.org/10.1038/srep45238).

26. Riccio M, Battiston B, Pajardi G, Corradi M, Passaretti U, et al. Efficiency of Hyaloglide in the prevention of the recurrence of adhesions after tenolysis of flexor tendons in zone II: a randomized, controlled, multicentre clinical trial. *J Hand Surg Eur Vol.* 2010;35(2):130-8. doi: [10.1177/1753193409342044](https://doi.org/10.1177/1753193409342044).
27. Ozgenel GY, Etöz A. Effects of repetitive injections of hyaluronic acid on peritendinous adhesions after flexor tendon repair: a preliminary randomized, placebo-controlled clinical trial. *Ulus Travma Acil Cerrahi Derg.* 2012;18(1):11-7. doi: [10.5505/TJTES.2011.95530](https://doi.org/10.5505/TJTES.2011.95530).



Efficacy of combined screening tests used in the first trimester in predicting adverse pregnancy outcomes

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■ MAIN POINTS

- First-trimester screening test parameters (PAPP-A, β -hCG, NT) show potential in predicting adverse pregnancy outcomes. Low PAPP-A levels were significantly associated with gestational diabetes mellitus, fetal growth restriction, and preeclampsia.
- NT values were significantly elevated in pregnancies complicated by gestational diabetes mellitus.
- Elevated β -hCG levels were significantly associated with preterm birth and placenta accreta spectrum or placenta previa.
- ROC analysis revealed predictive thresholds: PAPP-A <0.64 for preeclampsia (AUC=0.760), β -hCG >1.01 for PAS/PP (AUC=0.814), and NT >0.75 for GDM (AUC=0.588).
- PAPP-A emerged as the most consistent biomarker across multiple adverse pregnancy outcomes.

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■ ABSTRACT

Aim: This study aims to evaluate the predictive efficacy of first-trimester screening test parameters, including free β -human chorionic gonadotropin (β -hCG), pregnancy-associated plasma protein-A (PAPP-A), and nuchal translucency (NT), in identifying adverse pregnancy outcomes such as gestational diabetes mellitus (GDM), fetal growth restriction (FGR), preeclampsia, preterm birth, and placenta accreta spectrum (PAS) or placenta previa (PP).

Materials and Methods: A retrospective cohort analysis involved 776 pregnant women who underwent first-trimester screening tests between January 2023 and August 2024. Patients were categorized into two groups based on the presence or absence of pregnancy complications.

Results: Pregnancy complications were identified in 36.6% of participants, with GDM, FGR, and preterm birth being the most common. PAPP-A levels were significantly reduced in pregnancies complicated by GDM ($p=0.033$), FGR ($p=0.048$), and preeclampsia ($p=0.001$). NT values were notably elevated in GDM cases ($p=0.016$). Free β -hCG levels were significantly higher in preterm birth ($p=0.040$) and PAS/PP cases ($p=0.016$). ROC analysis revealed notable predictive thresholds: PAPP-A <0.64 for preeclampsia (AUC=0.760, $p=0.001$) and β -hCG >1.01 for PAS/PP (AUC=0.814, $p=0.016$).

Conclusion: First-trimester screening test parameters, particularly PAPP-A, NT, and β -hCG, exhibit potential in predicting adverse pregnancy outcomes. Reduced PAPP-A levels correlate with GDM, FGR, and preeclampsia, while elevated NT and β -hCG levels are associated with GDM and PAS/PP, respectively. Although these markers demonstrate promise, larger-scale prospective studies are needed to confirm their clinical utility and reliability in predicting pregnancy complications.

Keywords: First trimester screening, Pregnancy complications, Gestational diabetes mellitus, Preeclampsia, Fetal growth restriction, Placenta accreta spectrum

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■ INTRODUCTION

Pregnancy induces substantial physiological and biochemical alterations in the female body commencing with conception. The physiological pressure and stress induced by pregnancy may precipitate the early onset of chronic diseases in predisposed individuals. Increasing evidence indicates that pregnancy complications, including GHT, PE, GDM, preterm la-

bor, and FGR, can adversely influence maternal and fetal outcomes and may have enduring detrimental effects on maternal and fetal health post-pregnancy. Notwithstanding several studies, there remains inadequate data to anticipate and avert poor pregnancy outcomes [1,2].

First-trimester examination Aneuploidy screening tests are

the most prevalent assessments for predicting the delivery of a child with chromosomal abnormalities during early pregnancy and for forecasting potential adverse pregnancy outcomes. The procedure utilizes the characteristics of PAPP-A, free β -hCG, and fetal NT [3]. The application of multi-parameter tests to forecast adverse pregnancy outcomes relies on biomarkers and ultrasound results. Predicting problems during pregnancy is important for taking precautionary measures to reduce adverse outcomes, planning effective pregnancy follow-up, and managing care. It also involves referring high-risk pregnant women to specialized centers or experts.

While adverse pregnancy outcomes are classified as distinct clinical processes, it is a fact that they share common pathophysiological mechanisms. Aberrant placenta implantation is the common cause of FGR, preeclampsia, and placental diseases, including placenta previa and the accreta spectrum. GDM or maternal hyperglycemia is related with spontaneous abortion, macrosomia, congenital abnormalities, stillbirth, and maternal preeclampsia, similar to other adverse pregnancy outcomes [4]. PAPP-A, a component of this screening test, has been demonstrated in numerous studies to positively regulate insulin-like growth factors (IGF) and may be linked to GDM. Likewise, diminished levels of 1st Trimester PAPP-A have been correlated with abortion and low birth weight [5,6]. Similarly, data indicates that elevated free β -hCG levels in both the 1st and 2nd trimesters are correlated with preeclampsia, gestational hypertension, and fetal growth restriction [7,8].

Our study sought to examine the predictive efficacy of parameters utilized in the first trimester prenatal screening tests advised for all pregnant women concerning the spectrum of gestational hypertension, preeclampsia, gestational diabetes mellitus, fetal growth restriction, and placenta previa or accreta. We assert that our findings will assist doctors in forecasting these adverse pregnancy outcomes, which bear significant implications for both maternal and newborn health, and may enhance patient management.

■ MATERIALS AND METHODS

Study design

This retrospective cohort research encompassed patients who received first-trimester combined tests at Etlik City Hospital from January 2023 to August 2024. This study followed the Declaration of Helsinki on Research Involving Human Subjects and received approval from the hospital's Ethics Committee (approval number: AESH-EK1–2024-913). Due to the retrospective nature of the study data, informed consent was not acquired from the patients.

Study participants

The study included 776 participants who performed first-trimester combined tests. Patients who did not deliver at our facility were excluded from the study. Subsequently, patients

were categorized into a control group comprising individuals without pregnancy complications, based on diagnoses of GDM, FGR, preterm labor, polyhydramnios, oligohydramnios, GHT, threatened preterm labor, imminent abortion, fetal anomaly, abortion, preeclampsia, intrauterine fetal demise, and placenta accreta spectrum or placenta previa.

The first-trimester combined test, which includes PAPP-A, f- β hCG, and nuchal translucency (NT), is performed around 11–14 weeks of gestation in our clinic. Demographic, clinical, laboratory, and ultrasonographic data from the cases were retrospectively obtained using the hospital data management system.

Definition of pregnancy complications

Gestational diabetes mellitus

In our clinic, two different methods are used to diagnose GDM during pregnancy: a single-stage 75-gram oral glucose tolerance test (OGTT) or a two-stage process. In the single-stage method, the fasting plasma glucose value should be below 92 mg/dL, the 1st hour value should be below 180 mg/dL, and the 2nd hour value should be below 153 mg/dL in the 75-gram OGTT performed between 24–28 weeks of pregnancy. If any of these values are high, GDM is diagnosed.

The two-stage approach commences with the administration of a 50-gram OGTT. Consequently, patients exhibiting plasma glucose levels of 130 mg/dL or higher undergo a 100-gram OGTT. The reference values for this test are established as 95 mg/dL for fasting, 180 mg/dL for the first hour, 155 mg/dL for the second hour, and 140 mg/dL for the third hour. GDM is diagnosed if a minimum of two out of these four values exceed the designated threshold values. These methods seek to guarantee the precise and reliable diagnosis of gestational diabetes [9].

Gestational hypertension

Gestational hypertension is defined by the International Society for the Study of Hypertension in Pregnancy (ISSHP) as hypertension that manifests after the 20th week of gestation, lacking the distinctive features of preeclampsia. This diagnosis defines hypertension as a systolic blood pressure (SBP) of 140 mmHg or greater, or a diastolic blood pressure (DBP) of 90 mmHg or greater. The differentiating characteristic of preeclampsia is the lack of symptoms unique to the condition, such as proteinuria or organ dysfunction. Gestational hypertension is regarded as a significant issue impacting maternal health during pregnancy and necessitates vigilant monitoring [10].

Pre-eclampsia

Preeclampsia is a multifaceted condition that arises throughout gestation and is characterized by the emergence of novel symptoms. This syndrome is marked by hypertension, typically arising after the 20th week of gestation, accompanied by

different indicators of organ malfunction. Indicators of organ dysfunction encompass proteinuria, compromised renal or hepatic function, coagulopathy, or fetal growth limitation. Preeclampsia is a significant pregnancy problem that requires vigilant monitoring and management post-diagnosis, since it may result in serious health issues for both the mother and the infant [11,12].

Preterm birth

Preterm birth is described as the delivery of an infant prior to the conclusion of the 37th week of gestation. The precise process causing spontaneous preterm birth remains mostly unclear in many instances. This syndrome is believed to possess a complex composition. Factors contributing to preterm birth encompass inflammation, uteroplacental ischemia or bleeding, uteroplacental infections, uterine overdistension, stress, and various immune system-mediated mechanisms. This syndrome is regarded as a significant obstetric issue necessitating thorough assessment and a multidisciplinary strategy due to its intricate etiology [13].

Fetal growth restriction

The diagnosis of late-onset fetal growth restriction (FGR) was evaluated according to the Delphi Consensus Criteria. Accordingly, the diagnosis of fetal growth restriction was made when the abdominal circumference (AC) or estimated fetal weight (EFW) fell below the 3rd percentile. At least two additional criteria were required to make this diagnosis definitive. These criteria were as follows: (1) AC or EFW below the 10th percentile, (2) AC or EFW decreased by more than two quartiles, (3) Abnormalities were detected in Doppler ultrasound results. Among the Doppler abnormalities, the umbilical artery Doppler pulsatility index (PI) exceeding the 95th percentile or the cerebro-placental ratio (CPR) falling below the 5th percentile. The evaluation of these criteria together was applied to increase the accuracy of the FGR diagnosis [14].

Placenta accreta spectrum or placenta previa

Placenta previa refers to the presence of placental tissue that obstructs the internal cervical os. An unusual placental location may result in considerable antepartum, intrapartum, and/or postoperative uterine bleeding. Further sequelae include the requirement for cesarean delivery and an increased risk of preterm birth.

PAS denotes the abnormal infiltration of trophoblasts into the myometrium, sometimes reaching or surpassing the serosa. The clinical relevance is in the placenta's inability to detach spontaneously during delivery, with manual extraction efforts resulting in hemorrhage, which may be life-threatening and frequently necessitates hysterectomy. The pathogenesis of most cases of PAS is thought to include placental implantation at a location of compromised decidualization resulting from previous damage to the endometrial-

myometrial interface. The principal risk factor for PAS is the existence of placenta previa subsequent to a prior surgical section [15].

Statistical analysis

Statistical analyses were conducted utilizing IBM SPSS version 22.0 (IBM Corporation, Armonk, NY, USA). Normality of the distribution was assessed separately for each group. The Shapiro–Wilk test was applied for subgroups with $n < 50$, while the Kolmogorov–Smirnov test was used for larger groups ($n \geq 50$). Given that several adverse outcome subgroups had small sample sizes, primary comparisons were conducted using non-parametric tests (Mann–Whitney U), which are robust to non-normality. In addition, because no prospective sample size calculation was performed at study initiation, a post-hoc power analysis was performed using G*Power software (version xx, Universität Düsseldorf, Germany). Based on the observed effect sizes of our strongest associations (e.g., PAPP-A predicting preeclampsia, $AUC=0.760$; β -hCG predicting PAS/PP, $AUC=0.814$), the achieved power was calculated as 94.5% and 80.2%, respectively, at $\alpha=0.05$. Endpoints with smaller AUCs (~ 0.58 – 0.59) yielded more limited power (≈ 53 – 70%). Descriptive statistics for continuous variables are presented as "mean $\pm$ standard deviation" for normally distributed data, and as "median (interquartile range)" for non-normally distributed data. Categorical variables were analyzed using the chi-squared test or Fisher's exact test. Continuous variables, both normally and non-normally distributed, were analyzed using the independent sample t-test and the Mann-Whitney U test, respectively. The receiver operating characteristic (ROC) curve was utilized to compute and compare the areas under the curve (AUC) and establish the optimal cutoff values. Statistical significance for all tests was established as a P-value of less than 0.05.

RESULTS

The research examined the correlation between first-trimester screening metrics and adverse pregnancy outcomes. A total of 776 pregnancies were assessed, of which 492 (63.4%) were uncomplicated, whereas 284 (36.6%) experienced at least one problem. The prevalence of adverse pregnancy outcomes are summarized in Table 1 and depicted in Figure 1. Common problems comprised gestational diabetes mellitus (9.3%), fetal growth restriction (6.3%), and premature delivery (5.9%). Significantly, certain patients encountered multiple problems concurrently.

A comparison of first-trimester screening parameters β -hCG, PAPP-A, and NT between pregnancies with problems and those without showed no significant difference in the total group analysis (Table 2). Nonetheless, subgroup studies revealed significant results for some problems. β -hCG levels were markedly elevated in instances of preterm birth ($p=0.040$) and placenta accreta spectrum (PAS) or placenta

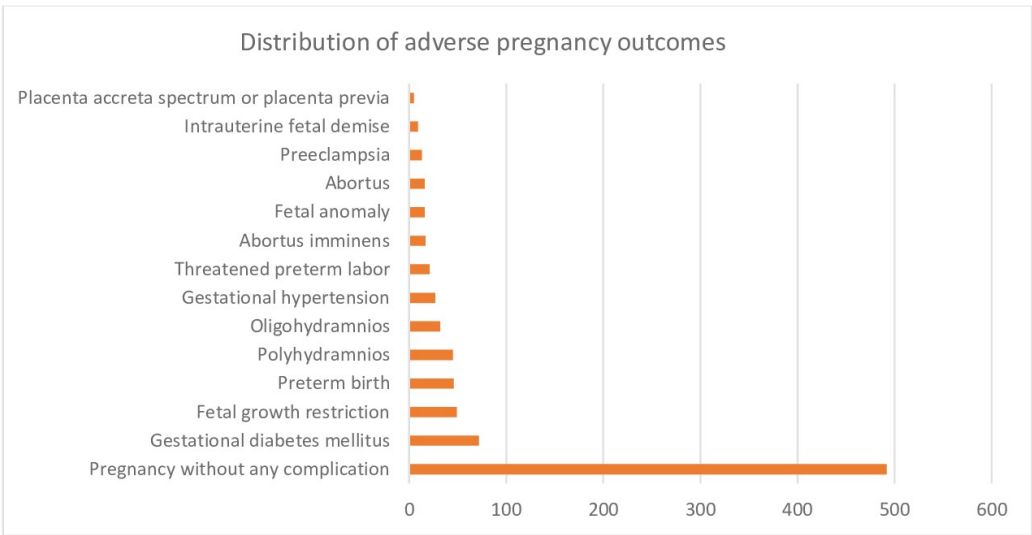


Figure 1. Distribution of adverse pregnancy outcomes.

previa (p=0.016) (Table 3). PAPP-A levels were markedly reduced in pregnancies affected by GDM (p=0.033), FGR (p=0.048), and preeclampsia (p=0.001) (Table 4). NT values were markedly elevated in pregnancies with gestational diabetes mellitus (p=0.016) (Table 5).

ROC analysis was conducted to evaluate the predictive significance of these indicators for specific problems (Table 6). Among the findings, NT with a cut-off value exceeding 0.75 predicted GDM with an area under the curve (AUC) of 0.588 (p=0.016). PAPP-A <0.79 correlated with gestational diabetes mellitus (AUC=0.578, p=0.033), while PAPP-A <0.82 forecasted fetal growth restriction (AUC=0.585, p=0.048). β -hCG >1.01 showed a moderate predictive value for PAS or placenta previa (AUC=0.814, p=0.016), but PAPP-A <0.64 exhibited significant predictive power for preeclampsia (AUC=0.760, p=0.001).

Table 1. Rate of adverse pregnancy outcomes observed.

Pregnancy without adverse outcomes	492 (63.4%)
Gestational diabetes mellitus	72 (9.3%)
Fetal growth restriction	49 (6.3%)
Preterm birth	46 (5.9%)
Polyhydramnios	45 (5.8%)
Oligohydramnios	32 (4.1%)
Gestational hypertension	27 (3.5%)
Threatened preterm labor	21 (2.7%)
Abortus imminens	17 (2.2%)
Fetal anomaly	16 (2%)
Abortus	16 (2%)
Preeclampsia	13 (1.6%)
Intrauterine fetal demise	9 (1.1%)
Placenta accreta spectrum or placenta previa	5 (0.6%)

*More than one pregnancy complication can occur simultaneously in the same patient. When calculating the rates associated with adverse pregnancy outcomes, each adverse outcome was calculated separately.

■ DISCUSSION

This study assessed the correlation between maternal free β -hCG, maternal PAPP-A, and NT values—parameters analyzed in the first trimester combined test—and various conditions including GDM, FGR, preterm labor, threatened preterm labor, GHT, preeclampsia, threatened miscarriage, intrauterine fetal demise, and placenta accreta spectrum or placenta previa. Maternal serum PAPP-A levels were dramatically reduced in individuals with GDM, FGR, and preeclampsia. We have found that NT MoM levels were markedly elevated solely in patients with GDM. We determined that free β -hCG maternal levels were markedly elevated in the cohort experiencing preterm labor and in patients with placenta accreta spectrum or placenta previa. These metrics are universally relevant across all centers, particularly those in poor countries, due to their simplicity and practicality.

The association between GDM, a prevalent perinatal complication, and PAPP-A, a parameter in the double test, has been extensively studied. PAPP-A levels have been demonstrated to be markedly reduced in GDM. Nonetheless, some research indicate that there is no correlation between dual test values and GDM [16,17]. A recent study by Yildiz et al. indicated that PAPP-A and Free β -hCG levels were markedly lower in the GDM group, but no significant change was observed for NT [3]. PAPP-A is a protease of insulin-like growth factor binding protein (IGFBP) that contributes to embryonic growth and development. Decreased PAPP-A levels are regarded as a marker of placental dysfunction and are correlated with FGR, preeclampsia, and chromosomal anomalies [18,19]. Simultaneously, research has corroborated the association between PAPP-A and metabolic disorders. Nonetheless, its association with GDM remains ambiguous. Kantomaa et al. conducted a study involving 4,697 pregnant women with GDM and discovered that levels of PAPP-A and

Table 2. Comparison of parameters used for first trimester screening in groups with and without adverse pregnancy outcomes.

	Pregnancy with complication n=284 (36.6%)	Pregnancy without complication n= 492 (63.4%)	p-value
β-hcg mom	0.83 (0.87)	0.81 (0.76)	0.982 ^a
PAPP-A mom	0.87 (0.69)	0.95 (0.71)	0.054 ^a
NT mom	0.76 (0.26)	0.72 (0.27)	0.102 ^a

^a: Mann-Whitney U, β-hcg: Beta-human chorionic gonadotropin, PAPP-A: Pregnancy associated plasma protein A, NT: Nuchal translucency, mom: A multiple of the median.

Table 3. Comparison of β-hCG used for first trimester anomaly screening in groups with adverse pregnancy outcomes and those without adverse pregnancy outcomes.

	β-hcg mom	p-value*
Pregnancy without adverse outcomes	0.81 (0.76)	
Gestational diabetes mellitus	0.78 (0.75)	0.158 ^a
Fetal growth restriction	0.79 (1.03)	0.991 ^a
Preterm birth	0.99 (1.1)	0.040^a
Polyhydramnios	0.87 (0.89)	0.288 ^a
Oligohydramnios	0.70 (0.88)	0.144 ^a
Gestational hypertension	0.85 (0.91)	0.529 ^a
Threatened preterm labor	0.89 (0.62)	0.713 ^a
Abortus imminens	0.82 (0.79)	0.787 ^a
Fetal anomaly	0.70 (0.64)	0.091 ^a
Abortus	0.71 (0.83)	0.078 ^a
Preeclampsia	0.82 (1.31)	0.729 ^a
Intrauterine fetal demise	0.84 (1.65)	0.724 ^a
Placenta accreta spectrum or placenta previa	1.68 (1.07)	0.016^a

* The given p values were found by comparing with the group that pregnancy without any adverse outcomes. β-hcg: Beta-human chorionic gonadotropin, mom: A multiple of the median.

Table 4. Comparison of PAPP-A used for first trimester anomaly screening in groups with adverse pregnancy outcomes and those without adverse pregnancy outcomes.

	PAPP-A mom	p-value*
Pregnancy without adverse outcomes	0.95 (0.71)	
Gestational diabetes mellitus	0.79 (0.58)	0.033^a
Fetal growth restriction	0.77 (0.67)	0.048^a
Preterm birth	0.88 (0.61)	0.713 ^a
Polyhydramnios	1.08 (0.67)	0.352 ^a
Oligohydramnios	0.85 (0.52)	0.134 ^a
Gestational hypertension	0.89 (0.69)	0.928 ^b
Threatened preterm labor	1.14 (1.11)	0.061 ^a
Abortus imminens	1.18 (1.07)	0.427 ^a
Fetal anomaly	0.89 (0.50)	0.284 ^a
Abortus	0.77 (1.83)	0.348 ^a
Preeclampsia	0.53 (0.35)	0.001^a
Intrauterine fetal demise	0.85 (0.95)	0.473 ^a
Placenta accreta spectrum or placenta previa	0.79 (0.38)	0.521 ^a

* The given p values were found by comparing with the group that pregnancy without any adverse outcomes. PAPP-A: Pregnancy associated plasma protein A, mom: A multiple of the median.

free β-hCG were dramatically decreased, while NT MoM levels were markedly elevated in the GDM cohort [20]. Conflicting data exist regarding the relationship between NT and GDM. One study examined NT between insulin-dependent and insulin-free GDM pregnant women, revealing no statistically significant difference [21]. A study comparing normal pregnant women and those with GDM revealed no statistically significant change in NT [17]. Our investigation found no statistically significant difference in β-hCG levels between the GDM group and the control group. Nonetheless,

PAPP-A levels were statistically considerably lower, while NT mom values were significantly greater exclusively in the GDM group among all adverse pregnancy outcomes.

Gestational hypertension and preeclampsia are significant contributors to maternal and neonatal morbidity and mortality, impacting 2-8% of pregnancies. Given the parallels in placental pathophysiology between FGR and the onset of GHT and preeclampsia, it is anticipated that analogous biomarkers will forecast both conditions [22]. A study revealed that PAPP-A and Free β-hCG levels were markedly reduced in pa-

Table 5. Comparison of NT used for first trimester anomaly screening in groups with adverse pregnancy outcomes and those without adverse pregnancy outcomes.

	NT mom	p-value
Pregnancy without adverse outcomes	0.72 (0.27)	
Gestational diabetes mellitus	0.78 (0.28)	0.016^a
Fetal growth restriction	0.74 (0.19)	0.999 ^a
Preterm birth	0.76 (0.35)	0.121 ^a
Polyhydramnios	0.75 (0.23)	0.266 ^a
Oligohydramnios	0.70 (0.22)	0.444 ^a
Gestational hypertension	0.78 (0.33)	0.546 ^a
Threatened preterm labor	0.68 (0.14)	0.388 ^a
Abortus imminens	0.80 (0.29)	0.114 ^a
Fetal anomaly	0.67 (0.21)	0.207 ^a
Abortus	0.66 (0.61)	0.795 ^a
Preeclampsia	0.78 (0.23)	0.247 ^a
Intrauterine fetal demise	0.81 (0.33)	0.267 ^a
Placenta accreta spectrum or placenta previa	0.76 (0.19)	0.631 ^a

^a The given p values were found by comparing with the group that without adverse pregnancy outcomes. NT: Nuchal translucency, mom: A multiple of the median.

Table 6. Evaluation of first trimester screening parameters in predicting adverse pregnancy outcomes using ROC analysis.

	LR+	Cut-off*	Sensitivity	Specificity	AUC	%95 CI	P-value
NT mom (for GDM)	1.37	>0.75	58.3%	57.4%	0.588	0.52 – 0.66	0.016
PAPP-A mom (for GDM)	1.28	<0.79	62.3%	51.4%	0.578	0.51 – 0.65	0.033
PAPP-A mom (for FGR)	1.44	<0.82	60.1%	58.3%	0.585	0.50 – 0.67	0.048
β-hcg mom (for PAS or Placenta previa)	2.13	>1.01	80%	62.6%	0.814	0.70 – 0.93	0.016
β-hcg mom (for preterm birth)	1.35	>0.94	80%	62.6%	0.592	0.50 – 0.68	0.040
PAPP-A mom (preeclampsia)	2.54	<0.64	78.4%	69.2%	0.760	0.62 – 0.90	0.001

*Cut-off values were found according to Youden index. LR: Likelihood ratio, AUC: Area under the curve, CI: Confidence Interval, β-hcg: Beta-human chorionic gonadotropin, PAPP-A: Pregnancy associated plasma protein A, NT: Nuchal translucency, mom: A multiple of the median, GDM: Gestational hypertension, FGR: Fetal growth restriction, PAS: Placenta accreta spectrum

tients with proteinuric GHT and FGR [23]. Tul et al. discovered that only low levels of PAPP-A were linked to FGR. Nevertheless, they asserted that all other indicators were ineffective in predicting GHT [16]. In a separate investigation, PAPP-A and Free β-hCG levels were dramatically decreased in patients with preeclampsia, FGR, and placental abruption [24]. D’Antonio et al. also identified markedly reduced PAPP-A levels in pregnancies complicated by preeclampsia, FGR and preterm birth [25]. PAPP-A is a metric utilized in the first trimester preeclampsia risk assessment approach employed by the Fetal Medicine Foundation (FMF) [26]. A guideline issued by the Royal College of Obstetricians and Gynaecologists in 2024 advised that patients with PAPP-A values below 0.415 mom should get closer monitoring and ultrasound follow-up for FGR [27]. In our investigation, consistent with the existing literature, only PAPP-A levels were significantly decreased in the FGR and preeclampsia cohorts. The disparity in free β-hCG and NT maternal levels was not statistically significant in these conditions The PAPP-A level, a parameter of the second trimester screening test, may serve as an additional finding or supportive data for selecting patients to initiate low-dose aspirin [28] which is utilized for preeclampsia prophylaxis and has recently received strong evidence-based recommendations.

Preterm delivery is a prevalent pregnancy condition associated

with negative mother and newborn outcomes. The incidence ranges from 7.8% to 12%, contingent upon the country, and results in several complications impacting around 15 million infants globally each year [29]. Numerous research has been undertaken to forecast preterm birth; however, no highly substantiated predictor has been identified to yet. The parameters of first-trimester combined tests have been extensively examined for predictive purposes, although the findings remain contentious. In a study, pregnant women who delivered at or before 34 weeks were compared with those who delivered at or after 37 weeks. Despite elevated levels of PAPP-A and Free β-hCG in the preterm birth cohort, this disparity was not statistically significant [16]. One study indicated that PAPP-A levels below 0.40 mom may be utilized for identifying FGR, preterm birth, and GHT [30]. Yildiz et al. discovered that only low PAPP-A levels were correlated with preterm labor [31]. Swiercz et al. demonstrated a link between Free β-hCG in the first trimester and low PAPP-A, as well as preterm birth [32]. In our investigation, preterm birth was statistically significant alone with elevated maternal β-hCG levels. The inconsistent results indicate that the use of these markers, whether individually or in combination, remains insufficiently accurate for predicting premature birth. More comprehensive research are required for this purpose.

Currently, the rising incidence of cesarean sections, coupled

with the escalation of placenta previa and accreta spectrum instances, remain a significant worry for obstetricians. Despite numerous studies indicating that ultrasound may diagnose these placental diseases in the first and early second trimesters, the inability to identify a substantial proportion of them during these gestational periods is seen as a considerable issue [33–35]. Our investigation did not identify a link between PAPP-A and the placenta accreta spectrum (PAS) or placenta previa (PP) as reported in prior research [36,37]. A 2019 study revealed a strong link between PAPP-A levels and the extent of bleeding in patients with PAS; however, it also demonstrated no correlation between β -hCG levels and PAS compared to the control group [38]. Buke et al. discovered that elevated PAPP-A and β -hCG levels in the first trimester correlated with PAS [39]. In another investigation, a difference in β -hCG levels was noted between the control group and the PP group; however, this difference was not statistically significant. When comparing the PAS group to the control group, the disparity in both biochemical parameters was statistically significant, with the mean values being elevated for the PP and PAS groups [40]. In our investigation, the elevation of β -hCG in mothers of PP and PAS patients was substantially different from that of the control group. Nonetheless, similar to other studies, the limited sample size in our research is a challenge, necessitating larger investigations with further meta-analyses to enhance the reliability of these data.

Limitations

This study has several limitations. Although the overall sample size was relatively large, the number of cases with some rare outcomes (such as preeclampsia and PAS/PP) was limited, which may have affected the statistical power of these subgroup analyses. Furthermore, since no prospective sample size calculation was performed at the beginning of the study, we conducted a post-hoc power analysis. This revealed adequate power for the strongest associations (94.5% for PAPP-A and preeclampsia; 80.2% for β -hCG and PAS/PP), while outcomes with modest AUC values had limited power (~53–70%). This limitation may explain why some results were not statistically significant and highlights the importance of larger, prospective studies. In addition, as this was a retrospective design, prospective power analysis could not be performed. Finally, only univariable ROC analyses were conducted; multivariable models could not be applied due to case distribution. Future larger, prospective studies with multivariable approaches are needed to validate and strengthen these findings.

CONCLUSION

This study investigated the possible implications of biochemical indicators (free β -hCG, PAPP-A) and NT values obtained in the first-trimester combined test in predicting adverse pregnancy outcomes. Our findings indicated that PAPP-A levels were markedly reduced in problems such as GDM, FGR, and

preeclampsia, whereas NT exhibited a considerable elevation correlated with GDM. Moreover, free β -hCG levels were considerably elevated in instances of preterm labor and PAS/PP cases. Consequently, the significance of forthcoming large-scale and high-evidence investigations is underscored to enhance the efficacy of these biochemical parameters in clinical applications. Screening test characteristics such as PAPP-A may be associated with certain adverse outcomes, but their incorporation into routine clinical practice requires confirmation in larger, prospective studies.

Ethics Committee Approval: This study followed the Declaration of Helsinki on Research Involving Human Subjects and received approval from the Etlik City Hospital's Ethics Committee (approval number: AESH-EK1–2024-913).

Informed Consent: Since the study was designed retrospectively, no written informed consent form was obtained from patients.

Peer-review: Externally peer-reviewed.

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REFERENCES

- Gogineni VSM, Manfrini D, Aroda SH, Zhang Y, Nelson DS, Egerman R, et al. Variations in Awareness of Association Between Adverse Pregnancy Outcomes and Cardiovascular Risk by Specialty. *Cardiol Ther.* 2021;10(2):577–92. doi: [10.1007/s40119-021-00220-y](https://doi.org/10.1007/s40119-021-00220-y).
- Young B, Hacker MR, Rana S. Physicians' knowledge of future vascular disease in women with preeclampsia. *Hypertens Pregnancy.* 2012;31(1):50–8. doi: [10.3109/10641955.2010.544955](https://doi.org/10.3109/10641955.2010.544955).
- Yildiz A, Yozgat ST, Cokmez H, Yildiz FŞ. The predictive value of the first trimester combined test for gestational diabetes mellitus. *Ginekol Pol.* 2023;94(5):395–399. doi: [10.5603/GP.a2022.0036](https://doi.org/10.5603/GP.a2022.0036).
- Chiefari E, Arcidiacono B, Foti D, Brunetti A. Gestational diabetes mellitus: an updated overview. *J Endocrinol Invest.* 2017;40(9):899–909. doi: [10.1007/s40618-016-0607-5](https://doi.org/10.1007/s40618-016-0607-5).
- Ruge S, Pedersen JF, Sørensen S, Lange AP. Can pregnancy-associated plasma protein A (PAPP-A) predict the outcome of pregnancy in women with threatened abortion and confirmed fetal viability? *Acta Obstet Gynecol Scand.* 1990;69(7-8):589–95. doi: [10.3109/00016349009028701](https://doi.org/10.3109/00016349009028701).
- Pedersen JF, Sørensen S, Ruge S. Human placental lactogen and pregnancy-associated plasma protein A in first trimester and subsequent fetal growth. *Acta Obstet Gynecol Scand.* 1995;74(7):505–8. doi: [10.3109/00016349509024379](https://doi.org/10.3109/00016349509024379).
- Wenstrom KD, Owen J, Boots LR, DuBard MB. Elevated second-trimester human chorionic gonadotropin levels in association with poor pregnancy outcome. *Am J Obstet Gynecol.* 1994;171(4):1038–41. doi: [10.1016/0002-9378\(94\)90030-2](https://doi.org/10.1016/0002-9378(94)90030-2).

8. Mallick MP, Ray S, Medhi R, Bisai S. Elevated serum β hCG and dyslipidemia in second trimester as predictors of subsequent Pregnancy Induced Hypertension. *Bangladesh Med Res Counc Bull.* 2014;40(3):97–101. doi: [10.3329/bmrcb.v40i3.25230](https://doi.org/10.3329/bmrcb.v40i3.25230).
9. ElSayed NA, Aleppo G, Aroda VR, Bannuru RR, Brown FM, Bruemmer D, et al. 2. Classification and Diagnosis of Diabetes: Standards of Care in Diabetes-2023. *Diabetes Care.* 2023;46(Suppl 1):S19–40. doi: [10.2337/dc23-S002](https://doi.org/10.2337/dc23-S002).
10. Burton GJ, Redman CW, Roberts JM, Moffett A. Pre-eclampsia: pathophysiology and clinical implications. *BMJ.* 2019;366:l2381. doi: [10.1136/bmj.l2381](https://doi.org/10.1136/bmj.l2381).
11. Tranquilli AL, Dekker G, Magee L, Roberts J, Sibai BM, Steyn W, et al. The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP. *Pregnancy Hypertens.* 2014;4(2):97–104. doi: [10.1016/j.preghy.2014.02.001](https://doi.org/10.1016/j.preghy.2014.02.001).
12. Seyhanli Z, Bayraktar B, Baysoz OB, Karabay G, Sucu ST, Ulusoy CO, et al. The role of first trimester serum inflammatory indexes (NLR, PLR, MLR, SII, SIRI, and PIV) and the β -hCG to PAPP-A ratio in predicting preeclampsia. *J Reprod Immunol.* 2024;162:104190. doi: [10.1016/j.jri.2023.104190](https://doi.org/10.1016/j.jri.2023.104190).
13. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet.* 2008;371(9606):75–84. doi: [10.1016/S0140-6736\(08\)60074-4](https://doi.org/10.1016/S0140-6736(08)60074-4).
14. Gordijn SJ, Beune IM, Thilaganathan B, Papageorgiou A, Baschat AA, Baker PN, et al. Consensus definition of fetal growth restriction: a Delphi procedure. *Ultrasound Obstet Gynecol.* 2016;48(3):333–9. doi: [10.1002/uog.15884](https://doi.org/10.1002/uog.15884).
15. Horgan R, Abuhamad A. Placenta Accreta Spectrum: Prenatal Diagnosis and Management. *Obstet Gynecol Clin North Am.* 2022;49(3):423–38. doi: [10.1016/j.ogc.2022.02.004](https://doi.org/10.1016/j.ogc.2022.02.004).
16. Tul N, Pusenjak S, Osredkar J, Spencer K, Novak-Antolic Z. Predicting complications of pregnancy with first-trimester maternal serum free-beta hCG, PAPP-A and inhibin-A. *Prenat Diagn.* 2003;23(12):990–6. doi: [10.1002/pd.735](https://doi.org/10.1002/pd.735).
17. Savvidou MD, Syngelaki A, Muhaisen M, Emelyanenko E, Nicolaides KH. First trimester maternal serum free β -human chorionic gonadotropin and pregnancy-associated plasma protein A in pregnancies complicated by diabetes mellitus. *BJOG.* 2012;119(4):410–6. doi: [10.1111/j.1471-0528.2011.03253.x](https://doi.org/10.1111/j.1471-0528.2011.03253.x).
18. Lawrence JB, Oxvig C, Overgaard MT, Sottrup-Jensen L, Gleich GJ, Hays LG, et al. The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy-associated plasma protein-A. *Proc Natl Acad Sci U S A.* 1999;96(6):3149–53. doi: [10.1073/pnas.96.6.3149](https://doi.org/10.1073/pnas.96.6.3149).
19. Kaijomaa M, Rahkonen L, Ulander V-M, Hämäläinen E, Alfthan H, Markkanen H, et al. Low maternal pregnancy-associated plasma protein A during the first trimester of pregnancy and pregnancy outcomes. *Int J Gynaecol Obstet.* 2017;136(1):76–82. doi: [10.1002/ijgo.12002](https://doi.org/10.1002/ijgo.12002).
20. Kantomaa T, Väärasmäki M, Gissler M, Ryyänen M, Nevalainen J. First trimester maternal serum PAPP-A and free β -hCG levels and risk of SGA or LGA in women with and without GDM. *BMC Pregnancy Childbirth.* 2024;24(1):580. doi: [10.1186/s12884-024-06786-4](https://doi.org/10.1186/s12884-024-06786-4).
21. Spencer K, Cowans NJ, Spencer CE, Achillea N. A re-evaluation of the influence of maternal insulin-dependent diabetes on fetal nuchal translucency thickness and first-trimester maternal serum biochemical markers of aneuploidy. *Prenat Diagn.* 2010;30(10):937–40. doi: [10.1002/pd.2589](https://doi.org/10.1002/pd.2589).
22. Kane SC, Costa F da S, Brennecke S. First trimester biomarkers in the prediction of later pregnancy complications. *Biomed Res Int.* 2014;2014:807196. doi: [10.1155/2014/807196](https://doi.org/10.1155/2014/807196).
23. Ong CY, Liao AW, Spencer K, Munim S, Nicolaides KH. First trimester maternal serum free beta human chorionic gonadotrophin and pregnancy associated plasma protein A as predictors of pregnancy complications. *BJOG.* 2000;107(10):1265–70. doi: [10.1111/j.1471-0528.2000.tb11618.x](https://doi.org/10.1111/j.1471-0528.2000.tb11618.x).
24. Ranta JK, Raatikainen K, Romppanen J, Pulkki K, Heinonen S. Decreased PAPP-A is associated with preeclampsia, premature delivery and small for gestational age infants but not with placental abruption. *Eur J Obstet Gynecol Reprod Biol.* 2011;157(1):48–52. doi: [10.1016/j.ejogrb.2011.03.004](https://doi.org/10.1016/j.ejogrb.2011.03.004).
25. D'Antonio F, Rijo C, Thilaganathan B, Akolekar R, Khalil A, Papageorgiou A, et al. Association between first-trimester maternal serum pregnancy-associated plasma protein-A and obstetric complications. *Prenat Diagn.* 2013;33(9):839–47. doi: [10.1002/pd.4141](https://doi.org/10.1002/pd.4141).
26. Poon LCY, Stratieva V, Piras S, Piri S, Nicolaides KH. Hypertensive disorders in pregnancy: combined screening by uterine artery Doppler, blood pressure and serum PAPP-A at 11–13 weeks. *Prenatal Diagnosis.* 2010;30(3):216–23. doi: [10.1002/pd.2440](https://doi.org/10.1002/pd.2440).
27. Morris RK, Johnstone E, Lees C, Morton V, Smith G, the Royal College of Obstetricians and Gynaecologists. Investigation and Care of a Small-for-Gestational-Age Fetus and a Growth Restricted Fetus (Green-top Guideline No. 31). *BJOG.* 2024;131(9):e31–e80. doi: [10.1111/1471-0528.17814](https://doi.org/10.1111/1471-0528.17814).
28. Chaemsaitong P, Sahota DS, Poon LC. First trimester preeclampsia screening and prediction. *Am J Obstet Gynecol.* 2022;226(2S):S1071–S1097.e2. doi: [10.1016/j.ajog.2020.07.020](https://doi.org/10.1016/j.ajog.2020.07.020).
29. Walani SR. Global burden of preterm birth. *Int J Gynaecol Obstet.* 2020;150(1):31–33. doi: [10.1002/ijgo.13195](https://doi.org/10.1002/ijgo.13195).
30. Kantomaa T, Väärasmäki M, Gissler M, Sairanen M, Nevalainen J. First trimester low maternal serum pregnancy associated plasma protein-A (PAPP-A) as a screening method for adverse pregnancy outcomes. *J Perinat Med.* 2022;51(4):500–509. doi: [10.1515/jpm-2022-0241](https://doi.org/10.1515/jpm-2022-0241).
31. Yıldız Ş, Sert ÜY, Bilir E, Türkgeldi E, Nas T. Prediction of Adverse Obstetric Outcomes by First Trimester Screening with Free β -hCG and PAPP-A: A Prospective Study of 889 Singleton Pregnancies. *JGON.* 2020;17(4):497–503. doi: [10.38136/jgon.760133](https://doi.org/10.38136/jgon.760133).
32. Swiercz G, Zmelonek-Znamirska A, Szwabowicz K, Armanska J, Detka K, Młodawska M, et al. Evaluating the predictive efficacy of first trimester biochemical markers (PAPP-A, β -hCG) in forecasting preterm delivery incidences. *Sci Rep.* 2024;14(1):16206. doi: [10.1038/s41598-024-67300-6](https://doi.org/10.1038/s41598-024-67300-6).
33. Zosmer N, Fuller J, Shaikh H, Johns J, Ross JA. Natural history of early first-trimester pregnancies implanted in Cesarean scars. *Ultrasound Obstet Gynecol.* 2015;46(3):367–75. doi: [10.1002/uog.14775](https://doi.org/10.1002/uog.14775).
34. Cali G, Forlani F, Foti F, Minneci G, Manzoli L, Flacco ME, et al. Diagnostic accuracy of first-trimester ultrasound in detecting abnormally invasive placenta in high-risk women with placenta previa. *Ultrasound Obstet Gynecol.* 2018;52(2):258–64. doi: [10.1002/uog.19045](https://doi.org/10.1002/uog.19045).
35. Jauniaux E, Bhide A. Prenatal ultrasound diagnosis and outcome of placenta previa accreta after cesarean delivery: a systematic review and meta-analysis. *Am J Obstet Gynecol.* 2017;217(1):27–36. doi: [10.1016/j.ajog.2017.02.050](https://doi.org/10.1016/j.ajog.2017.02.050).
36. Desai N, Krantz D, Roman A, Fleischer A, Boulis S, Rochelson B. Elevated first trimester PAPP-a is associated with increased risk of placenta accreta. *Prenat Diagn.* 2014;34(2):159–62. doi: [10.1002/pd.4277](https://doi.org/10.1002/pd.4277).
37. Lyell DJ, Faucett AM, Baer RJ, Blumenfeld YJ, Druzin ML, El-Sayed YY, et al. Maternal serum markers, characteristics and morbidly adherent placenta in women with previa. *J Perinatol.* 2015;35(8):570–4. doi: [10.1038/jp.2015.40](https://doi.org/10.1038/jp.2015.40).
38. Penzhoyan GA, Makukhina TB. Significance of the routine first-trimester antenatal screening program for aneuploidy in the assessment of the risk of placenta accreta spectrum disorders. *J Perinat Med.* 2019;48(1):21–6. doi: [10.1515/jpm-2019-0261](https://doi.org/10.1515/jpm-2019-0261).
39. Büke B, Akkaya H, Demir S, Sağol S, Şimşek D, Başol G, et al. Relationship between first trimester aneuploidy screening test serum analytes and placenta accreta. *J Matern Fetal Neonatal Med.* 2018;31(1):59–62. doi: [10.1080/14767058.2016.1275546](https://doi.org/10.1080/14767058.2016.1275546).

40. Thompson O, Otigbah C, Nnochiri A, Sumithran E, Spencer K. First trimester maternal serum biochemical markers of aneuploidy in pregnancies with abnormally invasive placentation. *BJOG*. 2015;122(10):1370–6. doi: [10.1111/1471-0528.13298](https://doi.org/10.1111/1471-0528.13298).