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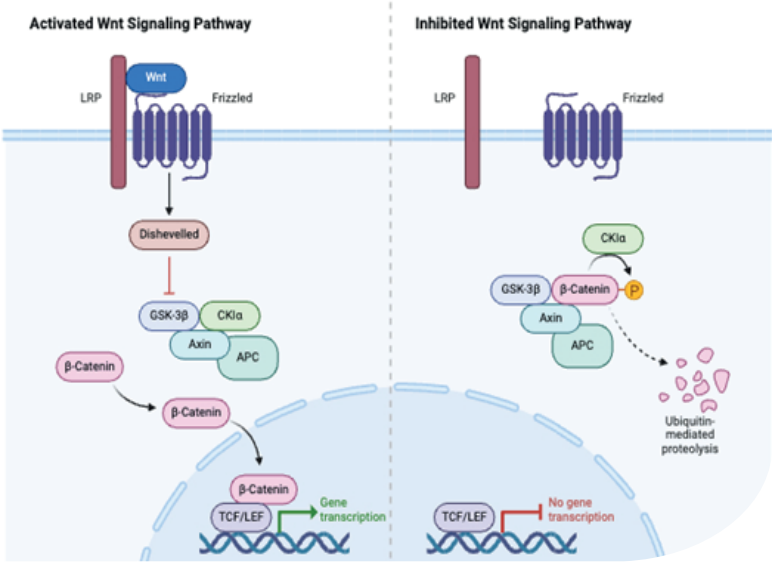
DEHM

Developments and Experiments in Health and Medicine

Volume:39 Issue:3 Year:2025
e-ISSN: 3062-2948



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Developments and Experiments in Health and Medicine (DEHM) is a peer reviewed academic journal, electronically published four-times (January, April, July and October) in a year.

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Developments and Experiments in Health and Medicine

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Developments and Experiments in Health and Medicine (ISSN: 1300-6622; e ISSN: 2602- 3148) is an international, double-blind peer-reviewed, open access publication of Dokuz Eylül University Faculty of Medicine which is published quarterly in January, April, July and October.
It publishes articles of original research conducted using scientific methods with appropriate hypotheses in all areas of medicine. In addition, it publishes reviews on current issues, rare medical cases, and letters to the editor containing the experiences and comments of specialist physicians in the field. Manuscripts are publishable in English. Developments and Experiments in Health and Medicine does not charge any fees to the author(s) for the evaluation and/or publication of submitted articles.
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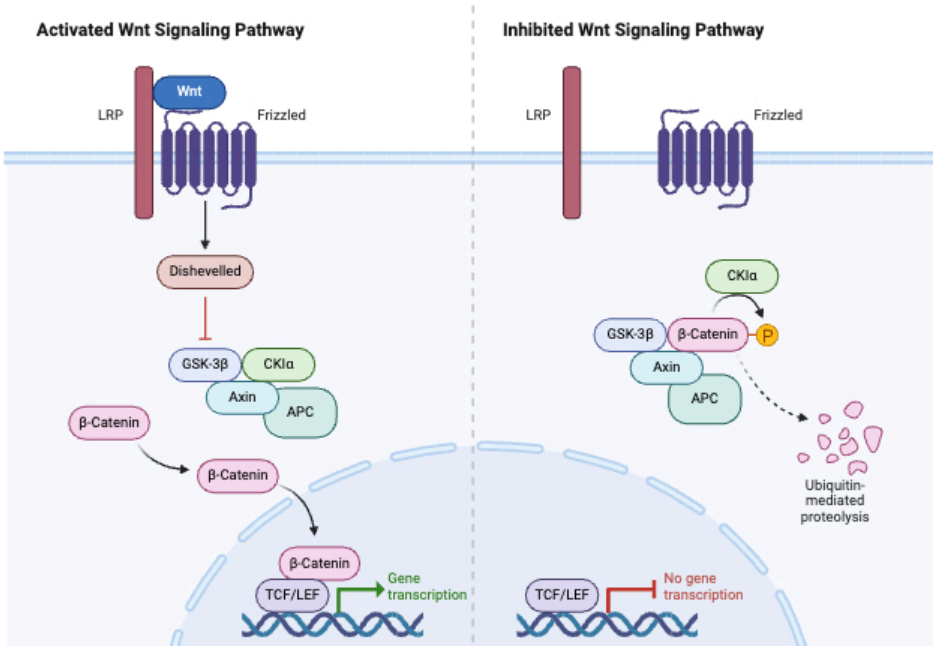

Review of carcinogenesis mechanisms and new therapeutic targets in colorectal cancer

Kolorektal kanserde karsinogenez mekanizmalarının ve yeni tedavi hedeflerinin gözden geçirilmesi
Ayşenur Başar ÖNOL, Volkan SEMİZ, Zekiye ALTUN

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Anti-Cancer treatments affecting PI3K/Akt/Mtor and Ras/MAPK pathways in neuroblastoma

Nöroblastomda PI3K/Akt/Mtor ve Ras/MAPK yolaklarını etkileyen anti kanser tedaviler

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Proteins in PI3K/Akt and Ras/MAPK Signaling in Neuroblastoma
Received: 19.12.2024
Accepted: 30.01.2025
Doi: 10.18614/dehm.1751044

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BACKGROUND
In this study, we applied combination therapy of RA and Tac to NB cells with different molecular properties and aimed to evaluate its effects on proliferation, differentiation, and apoptotic pathways in NB.

METHODS
Four cell lines of different characteristics; (KELLY, LAN-5, CHP-134, and SHSY5Y) were cultured and treated with various doses of RA and Tac. The IC50 values were determined by through WST analysis. The IC50 of the RA+Tac combination was applied to the cells. To determine the apoptosis/necrosis rate, the cells were dyed with Annexin V/PI. To examine the protein levels of certain pathways, Western Blot and IHC were performed.

RESULTS
The RA and RA+Tac treatments demonstrated beneficial effects in all the NB cell lines. The combination of RA+Tac treatments is relatively more efficient than RA in promoting apoptosis, inhibiting proliferation, and decreasing the expression levels of signal pathway proteins ($p < 0.05$). Only the Tac treatment did not have a significant effect on the NB cells. In low doses and in combination with RA, Tac was found to be effective on cells.

CONCLUSION
In summary, the NB cells differentiated with the RA treatment were more responsive when RA+Tac was administered. Tac exhibited a synergistic effect combined with RA and affected the crucial signal pathway proteins. Our studies lead to a more comprehensive study of the combination of RA and Tac.

KEYWORDS
Neuroblastoma, retinoic acid, signal pathways, treatment of neuroblastoma, tacrolimus

ÖZ
AMAÇ
Bu çalışmada, farklı moleküler özelliklere sahip Nöroblastom (NB) hücrelerinde Retinoik Asit (RA) ve Tacrolimus (Tac) kombinasyon tedavisi uygulandı ve NB'deki proliferasyon, diferansiyasyon ve apoptoz yolları üzerindeki etkilerin değerlendirilmesi amaçlandı.

YÖNTEM
Farklı özelliklere sahip dört hücre hattı; KELLY, LAN-5, CHP-134 ve SHSY5Y kültürlendi ve çeşitli dozlarda RA ve Tac ile tedavi edildi. IC50 değerleri WST analizi ile belirlendi. RA+Tac kombinasyonunun IC50 değeri hücrelere uygulandı. Apoptoz/nekroz oranını belirlemek için hücreler Annexin V/PI ile boyandı. Belirli sinyal yolaklarının protein seviyelerini incelemek için Western Blot ve IHC uygulandı.

SONUÇ
RA ve RA+Tac tedavisi, tüm NB hücre hatlarında yolak baskılayıcı etkiler gösterdi. RA+Tac tedavisinin kombinasyonu, apoptozu teşvik etme, proliferasyonu inhibe etme ve sinyal yolak proteinlerinin ekspresyon seviyelerini azaltma konusunda RA'dan nispeten daha etkilidir ($p < 0,05$). Sadece Tac tedavisinin NB hücreleri üzerinde anlamlı bir etkisi olmadı. Düşük dozlarda ve RA ile kombinasyon halinde, Tac hücreler üzerinde etkili bulundu. RA tedavisi ile farklılaşan NB hücreleri, RA+Tac uygulandığında tedaviye daha duyarlı hale geldi. Tac, RA ile kombine edildiğinde sinerjik bir etki gösterdi ve sinyal yolak proteinlerini etkiledi. Çalışmalarımız, RA ve Tac kombinasyonunun daha kapsamlı bir incelemesine yol açtı.

ANAHTAR KELİMELER
Nöroblastom, nöroblastom tedavisi, retinoik asit, sinyal yolakları

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Neuroblastoma (NB) is the most common extracranial solid malignancy of childhood (1). NB is responsible for approximately 15% of deaths related to cancer in children. While individuals with low- and intermediate-risk NB have a survival rate close to 100%, those with high-risk NB have a 5-year survival rate of less than 50% (2). The MYCN gene, located in the 2p24 region of the short arm of the second chromosome, plays an important role in the prognosis of NB. MYCN amplification NB is generally associated with the high-risk group, and survival in this group is not good despite intensive multimodal treatment. New chemotherapy regimes and molecular therapies are needed for effective treatment of advanced patients (3). Retinoic acid (RA) is a biologically active compound derived from vitamin A, an essential nutrient required for the proper functioning of the body. Especially during the embryonic period, RA helps cells grow and develop. Retinoids promote cellular differentiation and inhibit proliferation. Thus, it is regarded as a promising candidate for inhibiting the the progression of tumors (4).

Cell fate determination and differentiation is an important process for cells to function in specific tissues. As known, the differentiation process is impaired in NB cells (5). NB cells are derived from neural crest cells, and the differentiation ability of neural crest cells is impaired, so the cells are unable to become a mature cells. Neural crest cells give rise to stromal Schwann cells and neuroblastic cells, and these types of cells have varying degrees of differentiation in NB (6). As is well known, the degree of differentiation and tumor grade of Schwann cells is a predictive biomarker for NB. The PI3K/Akt/mTOR signal pathway plays an important role in the proliferation of NB. Pre-clinical and clinical studies have demonstrated that mTOR inhibitors, such as rapamycin and its variants, show long-term objective tumor response. Tacrolimus (Tac), a derivative of rapamycin, is being investigated as a drug that can modulate the PI3K/Akt/mTOR pathway, which is crucial for cellular proliferation (7). Tac has a negative effect on tumor growth by inhibiting the mTOR pathway (8). Simultaneously, Ras/MAPK pathway, involved in cellular death, differentiation, survival, and proliferation, is also a significant signaling pathway in pediatric solid tumors (9). It is necessary to interrogate new agents which may be effective against NB. There are no studies in the literature where RA and Tac are combined and applied to NB cells. In our research, we aimed to assess the effects of RA in combination with Tac on different types of NB cells. Apart from their use after organ transplantation, rapamycin and mTOR inhibitors are being commonly for treatment of cancer.

Materials and Methods

The following were used in our research: RPMI 1640 Media (Cegrogen), MTT (AppliChem), S-100 antibody (Santa Cruz, sc-53438), p44 antibody (Cell Signaling Technology, 9102), Akt antibody (Cell Signaling Technology, 9272), Ras antibody (Cell Signaling Technology, 3965), GAPDH antibody (Cell Signaling Technology, 97166), Ki67 antibody (Santa Cruz, sc-101861), Bcl-2 antibody (Santa Cruz, sc-65392), Caspase 3 antibody (Santa Cruz, sc-7272), BCA protein assay kit (Thermo Scientific), Annexin V/PI kit (BD Bioscience), Retinoic Acid (Sigma, R2625), Tacrolimus (MedChemExpress, HY-13756), and Cisplatin (MedChemExpress, HY-17394).

Cell culture and treatment

Different types of NB cell lines (KELLY, SHYS-5Y, LAN-5 and CHP-134) were cultured in RPMI 1640 medium containing 10% fetal bovine serum and 1% L-Glutamin+ antibiotic solution and maintained in a 5% CO2 incubator. The cells at 80% confluency were treated with increasing concentrations of RA (0.1 µM, 0.2 µM, 0.5 µM, 1 µM, 2 µM, 5 µM, and 10 µM), Tac (10 nm, 20 nm, 50 nm, 100 nm, 500 nm, 1000 nm, and 2000 nm), and combination doses of RA + Tac for 24, 48, and 72 hours.

Apoptosis assay

2 x 105 cells/well were seeded in six-well plates and treated with RA, Tac, RA+Tac, and Cisplatin (CDDP) for 48 hours. Then, cells were mechanically detached using cell scraper and harvested. The cell pellet dissociated with Binding Buffer (1X) and stained with 5 µL Annexin V and 10 µL PI dye for 15 minutes. Analyses were performed by using flow cytometry.

Immunocytochemistry

IHC was performed after the cells were treated with IC50 doses of drugs. Briefly, the cell pellet was obtained and dissociated with cell culture medium. 10 µL of cell suspension was dropped to the slide, and methanol was used to fixed the cells into the slide's surface. Then, cells were fix by incubating in 4% (v/v) paraformaldehyde in PBS for 20 minutes at room temperature. Slides were treated with the Antigen Retrieval Buffer at 95°C for 15 minutes. Cells were rinsed in PBS three times. 0.1% Triton X-100 in PBS was used to permeabilize the cells for 10 minutes at room temperature. Cells were rinsed in PBS three times. Then, blocking was performed by using 10% fetal bovine serum in PBS for 1 hour at room temperature. Cells were treated with the primary antibodies (S-100, Ki-67, Bcl-2, and Caspase-3) at a dilution of 1:200 overnight. Cells were

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rinsed in 1% goat serum in PBS 3 times for 10 minutes. Then, the secondary antibody was dropped to the slide's surface, covered with a coverslip and treated with the cells for an hour. Cells were rinsed in PBS three times. 1 drop of DAB chromogen solution and 1 drop of DAB enhancer were mixed and applied to the slide's surface and incubated for 15 minutes. Cells were rinsed in wash buffer three times. Slides were treated with hematoxylene for 5 minutes. Cells were rinsed in wash buffer three times. Slides were then treated for 5 minutes in increasing alcohol series (20%, 40, 60, 80%, and 100%). Then, slides were kept in xylol for an hour. Finally, slides were covered with a coverslip of an appropriate size. Slides were visualized under a light microscope.

Protein isolation from cells

NB cell lines were collected by centrifugation and washed with ice-cold PBS with 2 times. 1 mL of ice-cold RIPA lysis buffer for 1x107 cells was added to the cells and centrifugated at 13,000 x g for 20 minutes at 4°C. Then, the pellet was discarded, and the supernatant containing the protein was carefully collected in a new tube and kept on the ice for further analysis. BCA assay was performed to determine the protein concentration. Briefly, BSA standarts included in an assay was dissolved and prepared a series of standart solutions with known concentrations ranging from 0 to 2 mg/mL. 10 µL of each BSA standard solution and protein sample was added into separate wells of a 96-well microplate. 200 µL of the BCA working reagent was added to each well. Then, plate was incubated at 37°C for 30 minutes. After incubation, the absorbance was measured at 562 nm in a microplate reader.

Western blot

25 µg of isolated proteins were separated by 12% SDS-PAGE and transferred onto PVDF membranes. In the subsequent step, the membranes were blocked with 5% fat-free dry milk for 1 h at room temperature. The blots were incubated with Akt (1:1000), Ras (1:1000), p44 (1:1000) and GAPDH (1:1000) antibodies overnight at 4 °C. At room temperature, the membranes were incubated for 1 h with goat antirabbit IgG secondary antibodies conjugated to horseradish peroxidase (CST, 1:3000). The relative expression of each target protein was measured using GAPDH as an endogenous reference. The images were analyzed using the ImageJ 1.54 g program.

Statistical analysis

The data will be presented as mean ± standard deviation. Statistical analyses were performed using the SPSS 29.0 (IBM) software package at a significance level of $p < 0.05$. The conformity of the data to a normal distribution was assessed using the Kolmogorov-Smirnov test. The intergroup data were analyzed using the Kruskal-Wallis test, followed by the Mann-Whitney U test.

Results

Cell viability assay

Various doses of RA and Tac were administered to the cells. The viability assays of chemotherapeutics on KELLY, LAN-5, CHP-134, and SHSY5Y cells were evaluated using the MTT method at 24, 48, and 72-hour time points. RA showed a reduction effect on cell viability at a dose of 0.5 µm on all cells. At low doses, Tac does not affect viability. The Tac at a dose of 500 nm provided the IC50 value for all cell lines. RA treatment showed 38.7% ±1.8% cell viability after 48 hours (Figure 1a). Tac treatment demonstrated an average value of 33.7% ±1.2% cell viability during a period of 48 hours (Figure 1b). The levels of Tac and RA dosages were assessed using various combinations. The combination of RA and Tac (0.5 µm+500 nm) resulted in an IC50 value of 25.9% ± 1.7 after 48 hours of measurement (Figure 1c).

Apoptosis assay

During the early stages of apoptosis, the phosphatidylserine molecules on the cell surface are translocated to the outer surface of the membrane by a "flip-flop" movement in the membrane of an apoptotic cell. Annexin V is a protein that binds to phosphatidylserine molecules that are translocated outside the cell. Necrotic cells are marked with PI. Based on the results of the MTT results of drugs, the cells were treated for 48 hours with the IC50 concentration of RA, Tac and RA+Tac combination. Subsequently, cells were stained with Annexin V and PI to assess the percentages of apoptotic and necrotic cells. The BD Accuri C6 software was used to calculate and analyze all flow cytometry data. According to our findings, the rates of early+late apoptosis had similar rates among the cell lines treated with RA and RA+Tac. RA treatment was induced cell apoptosis on all cell lines. The rate of necrosis was low. The percentage of live cells in Tac treatment was higher than to other drug treatments. The synergistic effect of RA+Tac induced significant apoptosis. As a represented Figure 2a, apoptosis rate was relatively higher as a result of RA treatment, rather than RA+Tac combination on CHP-134 and SHSY-5Y cells. On the other hand, RA+Tac combination showed a slightly necrotic effect on cells. As shown in Figure 2b, RA+Tac combination had a comparatively higher apoptotic effect on LAN-5 and KELLY cells compared to RA treatment alone. (Figure 2).

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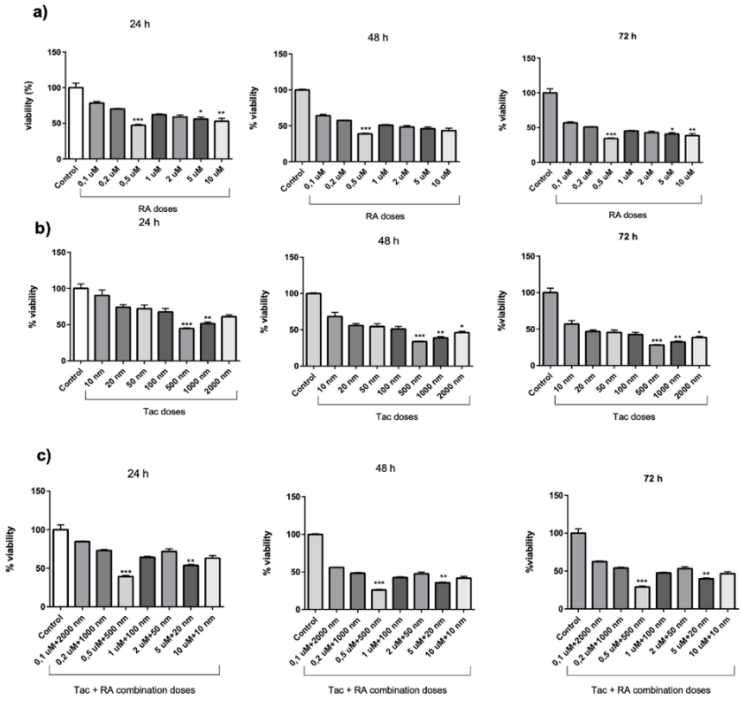

Figure 1. The cell viability of all chemotherapeutics: RA, Tac, and the combination of both agents. A various doses of all agent have administrated to all cell lines. As a result, a significant reduction in the number of viable cells after the treatment with RA, Tac, and their combination doses was found after 48 hours.

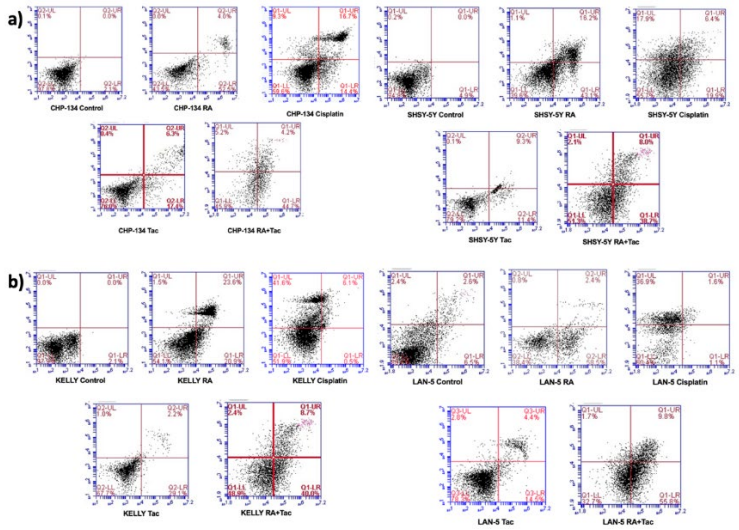

Figure 2. The apoptotic and necrotic effects of RA, Tac and combination. 2a. The CHP-134 and SHSY-5Y cell lines showed similar exposure to therapeutic agents in terms of apoptotic and necrotic cell death. 2b. The apoptotic effects of RA, Tac, and RA+Tac on KELLY and LAN-5 cell lines. RA exhibited an induction of proliferation on both cell lines. The apoptosis rate was relatively higher on LAN-5 with the treatment of both RA alone and RA+Tac combination compared to KELLY cell.

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Protein expressions

The expression of Ras, Akt, and p44 proteins was detected by Western Blot. RAS protein is widely involved in various physiological processes, such as proliferation, apoptosis, and cell survival. As known, RA prevents downregulation of RAS (10). In our study, after the drug treatment, the expression of the RAS protein in all the cell lines was analyzed. Compared to the control group, the expression of RAS protein in all cell lines was significantly reduced in both the RA and RA+Tac treatment groups, in all cell lines. The Tac treatment did not affect to lower the RAS levels. Compared to the control, RA+ Tac significantly decreased the Ras levels in all cell lines ($p < 0.001$) (Figure 3).

P44 is associated with MAPK pathway and is involved in many different physiological processes, such as inflammatory response, oxidative stress, and apoptosis (11). In our study, blotting analysis showed that RA treatment reduced the p44 levels . On SHSY-5Y and LAN-5 cells, RA decreased the p44 levels ($p < 0.05$). RA+Tac treatment is found relatively more effective on both cell lines ($p<0.01$). On KELLY and CHP-134 cells, RA inhibited the expression significantly ($p < 0.01$). RA+Tac treatment was relatively less effective than RA alone to reduce the expression ($p < 0.05$) (Figure 4a).

One more crucial protein investigated in the study was Akt. The activity of the protein p-Akt exhibited a notable reduction in all cell lines treated with RA and RA+Tac, compared to the control group. The resulting decrease was statistically significant ($p < 0.01$). A relative decrease was observed between the treatment groups of RA alone and RA+Tac ($p < 0.05$) (Figure 4b).

Immunocytochemistry

IC50 values of RA, Tac and RA+Tac were administered to NB cells. After the cells were collected, samples were fixated into the slide's surface. As shown in Figure 5a, S-100 differentiation marker exhibited a weak positivity in control group. The differentiating agents, RA and RA+Tac, showed more than 50% positive effect on cells. Ki67, a proliferation marker, was evaluated in NB cells (Figure 5b).

As a result of RA treatment, the proliferation rate was lower than the control group. The group treated with RA and RA+Tac had a significant reduction compared to the control ($p < 0.01$). There was no significant difference between the RA and RA+Tac groups ($p > 0.05$). The cellular expression of Caspase-3, a well-established indicator of programmed cell death, was evaluated (Figure 5c).

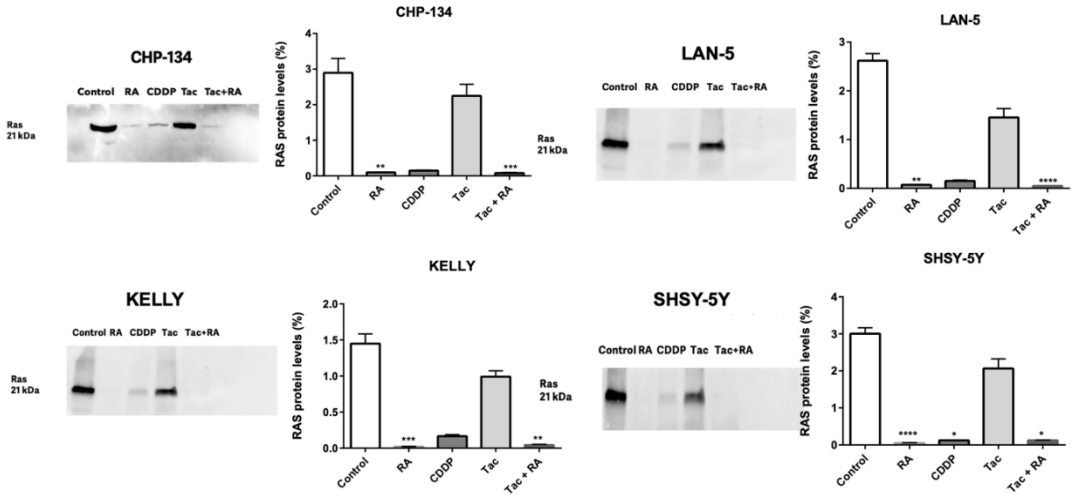

Figure 3. Ras protein levels determined by Western Blot. On all NB cell lines, RA and RA+Tac treatment significantly decreased the Ras levels.

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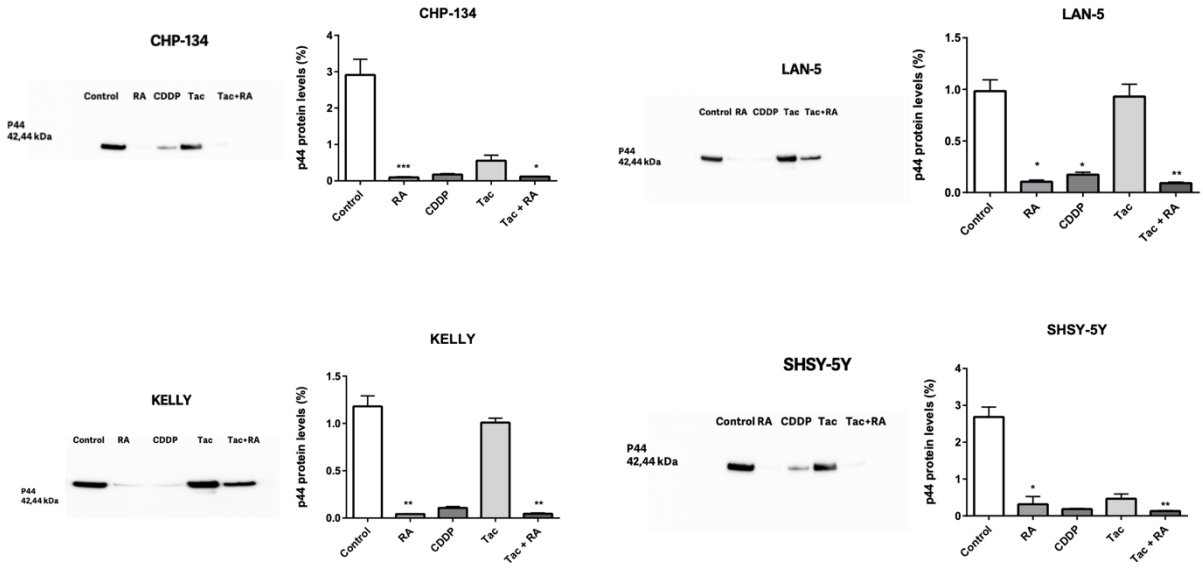

Figure 4. Determining the p44 levels by Western Blot. As shown, Tac was insufficient to suppress the protein expression. RA and RA+Tac treatment suppressed the p44.

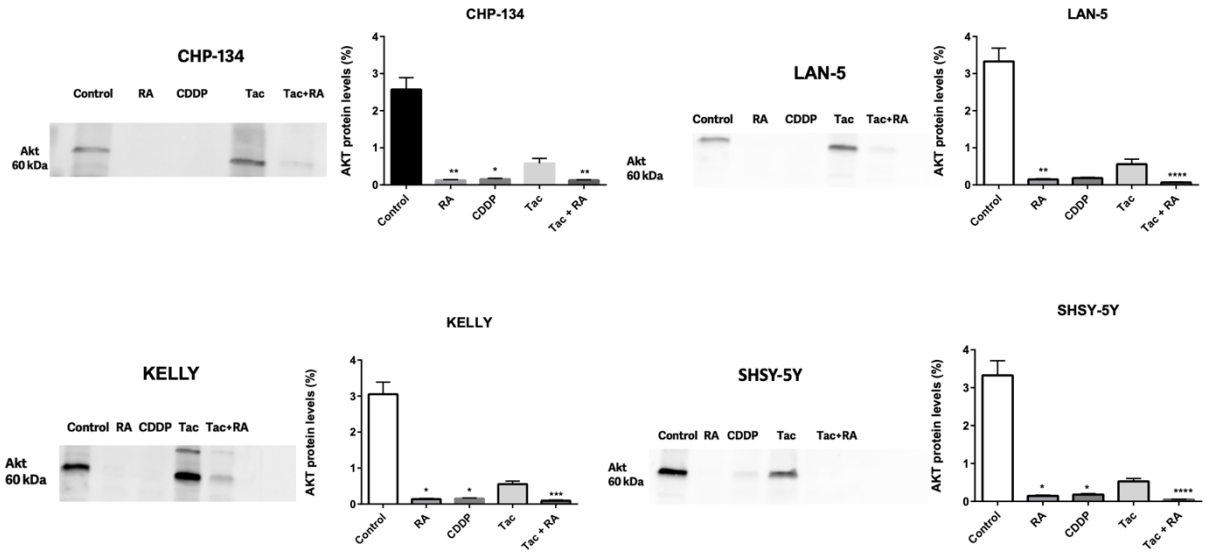

Figure 5. p-Akt levels explained by blotting. RA and RA+Tac treatment suppressed the protein expression.

The expression of Caspase-3 was significantly elevated in the groups that treated RA alone and RA+Tac. The two groups exhibited a statistically significant difference, compared to each other ($p < 0.05$). Tac alone did not induce apoptosis in NB cells. Bcl-2, a further mark of programmed cell death, is a biomarker that inhibits apoptosis. Immunocytochemical staining with Bcl-2 was performed (Figure 6d). As a result, the control group exhibited a high level of Bcl-2 expression. The expression level in Tac treatment group did not exhibit a significant decrease compared to the

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control group ($p > 0.05$). Bcl-2 showed very low positivity in RA treatment group. In RA+Tac group, Bcl-2 showed weak positivity.

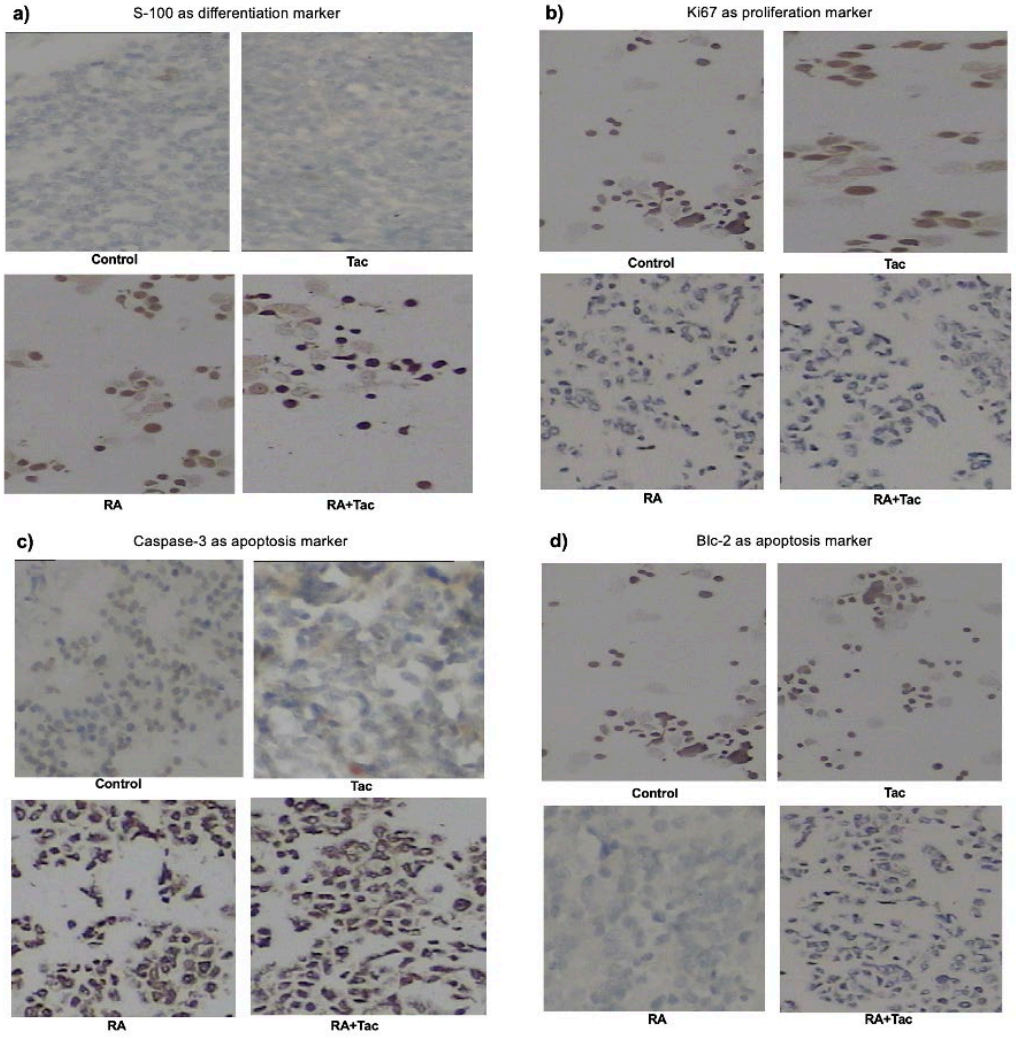

Figure 6. Differentiation, proliferation, and apoptosis biomarkers evaluated by immunocytochemistry. a) Weak positivity in control and Tac treatment. RA and RA+Tac treatment showed intense positivity in more than 50%. b) Ki67, a proliferation marker showed weak positivity resulting decreased expression levels in RA and RA+Tac treatment group. c) Caspase-3 exhibited strong positivity in RA and RA+Tac treatment, compared to control and Tac group. d) Bcl-2 is described as the anti-apoptotic protein. RA and RA+Tac showed weak positivity with Bcl-2. Tac showed a moderate positivity, compared to control. Immunocytochemical stainings, original magnification 100X.

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Discussion

As known, NB is a unique type of cancer characterized by newborns who may initially have either localized or metastatic disease (12). Interestingly, in some cases, NB might regress without any medical intervention.

However, older children can succumb to the disease after months to years of arduous therapy (13). Several biological factors contribute to the understanding of the clinical behavior in NB, such as histologic abnormalities, cytogenetic features, and molecular changes, specifically the amplification of the MYCN oncogene (14). Studies showed that treating the cells with retinoids can trigger the cells to differantiate, reprogram the enhancer landscape, and resulting in down-regulation of MYCN expression (15). Furthermore, some studies explain that the combination of MYCN inhibitors with RA suppresses the mTOR pathway and triggers apoptosis (16). In our study, we aimed to investigate the differentiation and apoptotic effect of RA on NB cells. To evaluate this, we used MYCN amplified cell lines; CHP-134, LAN-5, and KELLY and non-amplified cell; SHSY-5Y. Studies showed that treating MYCN amplified cell lines with RA induces differentiation in human NB cell lines (17,18). Similarly, we found a higher rate of Caspase-3 and S-100 expression after RA treatment, compared to the control group. In addition, the levels of Bcl-2, an anti-apoptotic biomarker, were reduced in the RA and RA+Tac treatments.

Tac is the best known mTOR inhibitor of rapamycin and a calcineurin inhibitor, it modulates mTOR in the absence of rapamycin with antiproliferative efficacy shown for many cancers (19). Tacrolimus with low doses has been found to trigger apoptosis and necrosis combined with mTOR inhibitors (20). In addition, Tac is currently used as the first-line immunosuppressant by organ transplant recipients in the clinical setting (21). A study demonstrated that Min6 cells treated to low concentrations of Tac exhibited an increased rate of apoptosis (22). Our findings showed that Tac treatment in NB cell lines had a significant impact on apoptotic indicators. However, by combining Tac with RA, the apoptotic effect was further enhanced. Therefore, we suggest that Tac and RA exhibit a synergistic impact on each other, depending on the dosage.

Ras/ERK and PI3K/Akt/mTOR signaling pathways are the main mechanisms of the cell to control cellular survival, differentiation, proliferation, metabolism, and motility. The PI3K/Akt/mTOR pathway is responsible to cell division, metabolism, and survival. Akt protein is an important molecule in cellular phenomena, many different intra-cellular processes including survival and proliferation in NB (23). We also performed Western blot to evaluate the p-Akt, Ras, and p44 levels. We found that the level of Akt expression in Tac treatment was lower compared to the control group. However, the combination of Tac and RA exhibited the most significant effect. A significant decrease was noted in the cells that administered only- RA in comparison to the control. Our findings indicated that Tac had a moderate level of success in reducing p-Akt levels. Tac has a similar effect on p-Akt levels, according to a study in the literature (22). Our investigation correlates with the current research in this field.

Presently, several genetic characteristics, such as RAS mutations, are being utilized as focusing points for the discovery of novel treatments for NB patients (9). Mutations affecting the RAS–MAPK pathway frequently occur in relapsed NB tumors, which suggests that activation of this pathway is associated with a more aggressive phenotype (24). p44/42 mitogen-activated protein kinase (MAPK), also named extracellular signal-regulated protein kinases (ERK1/2) is over expressed in various cancers including NB. p44 is associated with Ras protein in a downstream signal cascade (25). Studies have shown that the expression of RAS protein decreases in cells induced by RA (26). Similarly, our data demonstrated a significant decrease in expression of Ras in RA and RA+Tac. A similar study noted that RA affects the PI3K, ERK1/2, and p44/42 proteins (27). Tac had no effect in establishing an impact on the Ras mechanism. However, the combination group exhibited notable effect on Ras levels. Although Tac treatment exhibited high levels of p44, an important decreasing was found in RA and RA+Tac groups. It is evident that Tac is unable to independently affect the Ras-MAPK-ERK pathway.

Conclusion

To sum up, RA treatment regulates the differentiation, proliferation, and programmed cell death of NB cells. Tac is a calcineurin inhibitor that has relatively little effect on the signal pathways in cancer cells, as reported in the literature. This study demonstrated that Tac by itself was not effective in treating NB. However, combined with RA, Tac had a synergistic impact and altered crucial signal pathways in NB. Additional investigation is needed to be conducted to explore the pharmacology and impact of Tac in cancer. Since this study is done in vitro, additional analysis is necessary.

Author contributions

O.G, S.A, Z.A, and N.O designed the research. O.G and A.E performed the cell culture experiments. O.G, and A.E performed the Western Blot experiments. O.G performed the flow cytometry. O.G and A.E performed the immunocytochemistry staining. O.G and S.A performed the

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statistics. All the authors have equal contributions to the paper. All the authors contributed to the data interpretation and writing.

Funding

This project has been funded by Turkish Pediatric Oncology Group (TPOG).

Data availability

The data sets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

Declarations

Competing interests

The authors have no relevant financial or non- financial interests to disclose.

Ethical standarts

The study was approved by the Ethics Committee of the Dokuz Eylül University, Türkiye with the license number 2018/04-14. All aspects of this study, were performed in accordance with the principles of the Declaration of Helsinki (64th, 2013).

Informed consent

All participants gave their informed consent prior to their inclusion in the study.

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Assessment of intern doctors' attitudes towards ageism and related factors: A single-center cross-sectional study

Intörn doktorların yaş ayrımcılığına yönelik tutumlarının ve ilişkili faktörlerin değerlendirilmesi: Tek merkezli kesitsel bir çalışma

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Intern Doctors And Ageism
Received: 28.01.2025
Accepted: 24.02.2025
Doi: 10.18614/dehm.1751123

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BACKGROUND
It is well known that ageism, or discrimination against older individuals, is a pervasive issue in healthcare settings, potentially affecting the quality of care provided to this population. In this paper, it was aimed to assess the attitudes of intern doctors toward ageism and to identify the sociodemographic, educational, and experiential factors influencing these attitudes.

MATERIAL and METHODS
A cross-sectional study was conducted among 158 intern doctors at Dokuz Eylül University Faculty of Medicine during the 2024–2025 academic year. Data were collected utilizing the Sociodemographic Information Form and the Turkish version of the UCLA Geriatrics Attitudes Scale (UCLA-GA). Multiple linear regression analyses were executed to identify factors associated with UCLA-GA scores.

RESULTS
The mean total UCLA-GA score of participants was 47.5 ± 4.6, indicating generally positive attitudes toward geriatrics. Intern doctors who expressed a preference to live with their parents in the future showed significantly higher total UCLA-GA scores (β = 1.793, 95% CI: 0.198–3.388, p = 0.028). Additionally, willingness to work in institutions providing elder care services was significantly associated with higher scores in the Medical Care (β = 1.078, 95% CI: 0.255–1.900, p = 0.011) and Resource Distribution (β = 1.057, 95% CI: 0.241–1.873, p = 0.011) subscales. Notably, aging was predominantly associated with negative themes such as illness, dependency, and death.

CONCLUSIONS
As a result, intern doctors indicated generally positive attitudes toward ageism. Those who preferred to live with their parents in the future exhibited more favorable perceptions of aging, while willingness to work in elder care institutions was associated with higher scores of medical care and resource distribution subscales. Despite these positive perspectives, aging remains largely perceived through negative lenses, such as illness, dependency, and mortality, emphasizing the need for targeted educational interventions to foster a more balanced and nuanced understanding of aging among future healthcare professionals.

KEYWORDS
Ageism, attitude, intern doctors, UCLA-GA

ÖZ
AMAÇ
Yaş ayrımcılığı, yaşlı bireylere yönelik ayrımcılık, sağlık hizmetlerinde yaygın bir sorun olarak bilinmektedir ve bu durum, bu popülasyona sunulan bakımın kalitesini potansiyel olarak etkileyebilir. Bu çalışmada, intörn doktorların yaş ayrımcılığına yönelik tutumlarının değerlendirilmesi ve bu tutumları etkileyen sosyodemografik, eğitimsel ve deneyimsel faktörlerin belirlenmesi amaçlanmıştır.

GEREÇ VE YÖNTEM
Kesitsel bir çalışma, 2024-2025 akademik yılında Dokuz Eylül Üniversitesi Tıp Fakültesi'nde 158 intörn doktor arasında gerçekleştirilmiştir. Veriler, Sosyodemografik Bilgi Formu ve UCLA Yaşlılık Tutum Ölçeği'nin (UCLA-GA) Türkçe versiyonu kullanılarak toplanmıştır. UCLA-GA puanları ile ilişkili faktörleri belirlemek için çoklu doğrusal regresyon analizleri yapılmıştır.

BULGULAR
Katılımcıların toplam UCLA-GA puan ortalaması 47,5 ± 4,6 olarak hesaplanmış ve bu durum, yaşlılığa yönelik genel olarak olumlu tutumları göstermektedir. Gelecekte ebeveynleriyle birlikte yaşama isteğini ifade eden intörn doktorlar, toplam UCLA-GA puanlarında anlamlı derecede daha yüksek puanlara sahiptir (β = 1,793, %95 GA: 0,198–3,388, p = 0,028). Ayrıca, yaşlı bakım hizmeti veren kurumlarda çalışmaya istekli olma, Tıbbi Bakım (β = 1,078, %95 GA: 0,255–1,900, p = 0,011) ve Kaynak Dağılımı (β = 1,057, %95 GA: 0,241–1,873, p = 0,011) alt ölçeklerinde daha yüksek puanlarla anlamlı şekilde ilişkilendirilmiştir. Dikkat çekici bir şekilde, yaşlanma genellikle hastalık, bağımlılık ve ölüm gibi olumsuz temalarla ilişkilendirilmiştir.

SONUÇ
Sonuç olarak, intörn doktorlar yaşlılığa karşı genel olarak olumlu tutumlar gösterdiler. Gelecekte ebeveynleriyle yaşamayı tercih edenler yaşlanmaya ilişkin daha olumlu algılar sergilerken, yaşlı bakım kurumlarında çalışma isteği daha yüksek tıbbi bakım ve kaynak dağıtım alt ölçekleriyle ilişkilendirildi. Bu olumlu bakış açılarına rağmen, yaşlanma büyük ölçüde hastalık, bağımlılık ve ölüm gibi olumsuz merceklerden algılanmaya devam ediyor ve gelecekteki sağlık profesyonelleri arasında yaşlanmaya ilişkin daha dengeli ve ayrıntılı bir anlayış geliştirmek için hedefli eğitim müdahalelerine olan ihtiyacı vurguluyor.

ANAHTAR KELİMELER
Intörn doktorlar, tutum, UCLA-GA, yaş ayrımcılığı

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Ageism, defined as prejudice or discrimination based on a person's age, is an increasingly recognized issue in healthcare settings (1–4). With the global population aging at an unprecedented rate, the World Health Organization has emphasized the need to address ageism to ensure equitable healthcare delivery (1-5). Older individuals often present with complex health conditions, making them vulnerable to not only physiological challenges but also social and psychological barriers (6). Consequently, the quality of care provided to older adults can be influenced by healthcare professionals' attitudes toward this population (7). Understanding and mitigating ageist attitudes is, therefore, critical in training the next generation of physicians who will be responsible for managing the growing demographic of older patients (8).

Intern doctors, situated at the junction between undergraduate medical education and independent clinical practice, play a pivotal role in shaping the future landscape of patient care (9). Their attitudes towards older adults can be influenced by factors such as personal beliefs, prior experiences, and the quality of elderly training they receive (10). Negative stereotypes or misconceptions about aging may deter them from delivering compassionate care or engaging in the field of geriatrics (11). Conversely, positive attitudes can foster empathetic communication, accurate assessments, and improved therapeutic relationships, all of which are essential for optimizing health outcomes among the elderly (12).

Prior research has highlighted the influence of medical curricula, mentoring, and clinical exposure on shaping attitudes toward elderly patients (9). Studies indicate that structured education in gerontology and increased clinical interactions with older adults can effectively reduce ageist attitudes among trainee doctors (12). Despite these findings, there remains variability in the rigor and depth of elderly instruction across medical schools (10). Moreover, cultural, societal, and institutional factors can further modulate how intern doctors perceive and interact with elderly patients, pointing to the need for context-specific investigations (9). Of note, multiple studies found that medical students and junior doctors generally held positive to moderately positive attitudes toward older people (10,13,14). However, some research indicated slightly negative attitudes or ageism among medical trainees (13,15). Factors associated with more positive attitudes included elderly education or rotations (12,13), being a doctor rather than a nurse (16), and having caregiving experiences with older adults (15). Conversely, factors linked to more negative attitudes included being male, longer years of medical training, and lack of elderly care experience (15).

The present cross-sectional study aimed to investigate the intern doctors' attitudes toward ageism and to identify the sociodemographic, educational, and experiential factors that contribute to these attitudes. By examining a cohort of medical interns, this research seeks to clarify the extent to which current training protocols and personal backgrounds shape their perspectives on older adults. Identifying modifiable contributors to ageist attitudes will not only inform curriculum development but also provide a basis for interventions targeting attitudinal shifts in emerging healthcare professionals.

Materials and Methods

Study design, setting and ethical approval

This single-center cross-sectional study was conducted at Dokuz Eylül University (DEU) Faculty of Medicine. All data were collected during the 2024–2025 academic year. The target population of the study was determined as 287 intern doctors studying from the 2024–2025 academic year at Dokuz Eylül University Faculty of Medicine. Of these, 96 individuals either did not agree to participate in the study or did not provide informed consent. Additionally, data forms from 33 individuals were excluded because more than half of the required information was incomplete. In terms of inclusiveness, 55.05% of the target group was represented in this study. A flowchart showing the identification process of participants in the study is summarized in Figure 1.

During the data collection process, surveys were sent to intern doctors at Dokuz Eylül University Faculty of Medicine online through internship representatives. Initially, 287 intern doctors from the 2024–2025 academic year were considered the target population.

The research adhered to the principles outlined in the Declaration of Helsinki and received approval from the Institutional Review Board of the Dokuz Eylul University Faculty of Medicine Non-Interventional Research Ethics Committee (Decision No. 2024/27-06, dated 07/08/2024).

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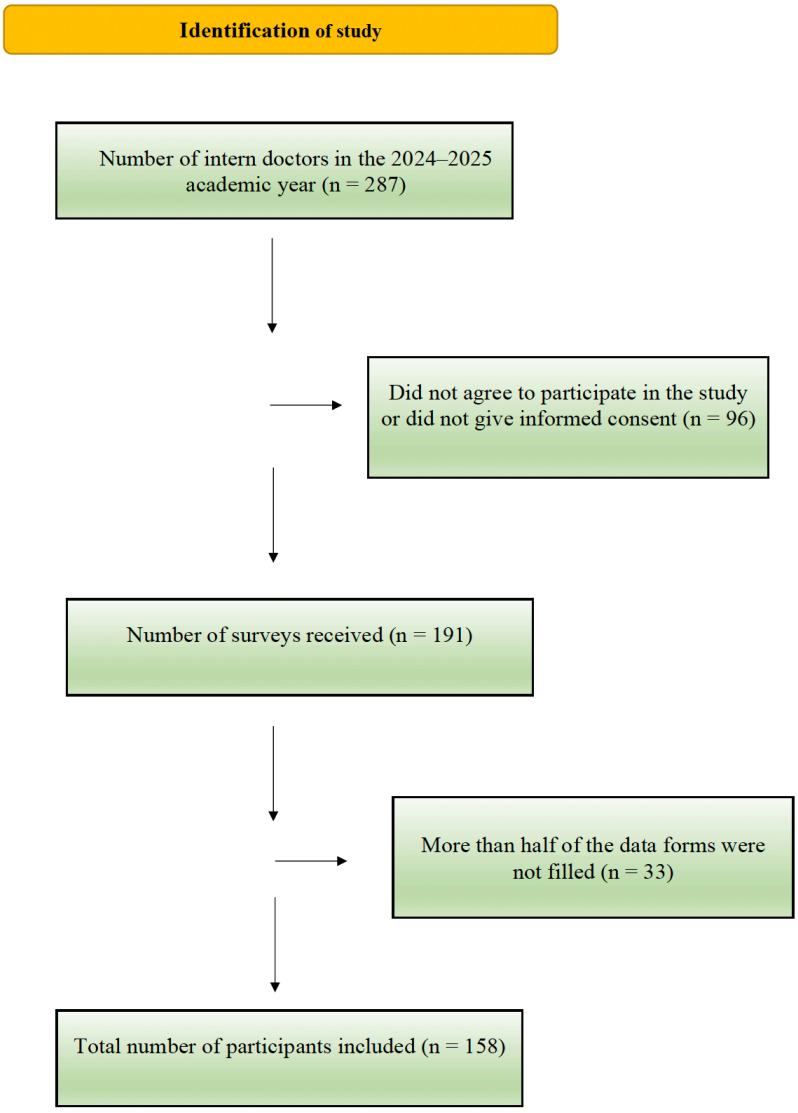

Figure 1. Flowchart showing the identification process of participants in the study

Study population, sampling and inclusion and exclusion criteria

The study population consisted of all intern doctors enrolled at DEU Faculty of Medicine during the specified academic year. Inclusion criteria were: (1) being an intern (final-year medical student) at DEU Faculty of Medicine in the 2024–2025 academic year, and (2) voluntarily agreeing to participate in the study. Interns who did not complete more than half of the data form and did not give consent for the study were excluded from the study. After excluding any incomplete or invalid responses, the final sample was determined.

Data collection instrument and data collection procedures

The Sociodemographic Information Form and the UCLA Geriatrics Attitudes (UCLA-GA) Scale were utilized in this study to assess demographic characteristics and attitudes toward elderly. The Sociodemographic Information Form gathered information regarding participants' gender, living

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arrangements, parental education level, family structure, and perceptions of income status, as well as their attitudes towards aging and elder care. The UCLA-GA Scale was employed to evaluate attitudes toward elderly individuals, focusing on aspects such as emotional responses, beliefs, and behaviors. Both the Sociodemographic Information Form and the UCLA-GA Scale were administered online to the participants, ensuring efficient distribution and data collection.

In the present study, the dependent variable is interns' attitudes toward ageism, as measured by the UCLA-GA Scale. The independent variables comprise the participants' sociodemographic characteristics, whether they live with older individuals, their preferences concerning future cohabitation or professional engagement with older adults, and any previous involvement in elderly health projects.

The sociodemographic information form

It was designed to ascertain participants' gender (female or male), their current living arrangement (living with family, in a dormitory, or elsewhere), and their parents' educational attainment (illiterate, literate, primary school, middle school, high school, or university and above). It additionally recorded the mother's and father's birth dates and inquired about the participant's family structure (nuclear, extended, single-parent, or other), as well as their perception of the family's income relative to expenses (higher than, equal to, or lower than expenses). The form also sought to capture the first concept that comes to mind when thinking about old age, whether the participant had ever lived with an older family member, and whether they would prefer to live with their parents in the future. Lastly, the form investigated participants' willingness to work in institutions providing elder care services (such as nursing homes or rehabilitation centers), and for those who were unwilling, it requested an explanation of their reasons.

UCLA Geriatrics Attitudes (UCLA-GA) scale

The UCLA Geriatrics Attitudes (UCLA-GA) Scale was originally developed in 1998 by Reuben and colleagues (17). It comprises a relatively small number of items, is multidimensional, and was validated in English using data from medical students and healthcare providers. Sahin et al. (2011) (18) conducted the Turkish validity and reliability study for the 14-item version of the scale, reporting a Cronbach's alpha of 0.67, which was deemed satisfactory. Tuckey's additivity test ($F=85.25$, $p < 0.0001$) further indicated that the scale items possess additive properties. The scale consists of four subdimensions—Social Values (SV), Medical Care (MC), Compassion (CP), and Resource Distribution (RD)—and is recommended for assessing attitudes toward older adults among those who provide elderly healthcare services, particularly given its concise and clear structure in the Turkish version. In its Turkish version, the UCLA-GA Scale consists of 14 items, each rated on a five-point Likert scale from 1 (strongly disagree) to 5 (strongly agree). Consequently, the lowest possible total score on the scale is 14, while the highest possible score is 70. Higher scores generally indicate more favorable or positive attitudes toward older adults. Medical care, Compassion, and Resource Distribution each contain four items, yielding possible subdimension scores ranging from 4 to 20, while Social value each consist of two items, with subdimension scores ranging from 2 to 10.

Statistical analysis

For continuous variables, The assumptions of normality and homogeneity of variances were checked using the Kolmogorov-Smirnov test and Levene's test, respectively. Descriptive statistics were calculated to summarize participants' demographic characteristics and their UCLA-GA scores. Continuous variables were expressed as means and standard deviations (mean ± SD), while categorical variables were presented as frequencies and percentages. Comparisons of UCLA-GA subscale and total scores according to categorical variables (e.g., gender, living arrangement, parental educational attainment, and willingness to work in elderly care institutions) were performed utilizing the independent samples t-test or one-way analysis of variance (ANOVA), depending on the number of groups being compared. For variables with significant group differences in ANOVA, post hoc Bonferroni tests were conducted to identify specific group differences. A multiple linear regression model was executed to evaluate the causal effects of independent variables on the dependent variable. The multiple linear regression analysis employed a backward stepwise method, with standardized and unstandardized coefficients beta and their corresponding 95% confidence intervals (CIs) for beta calculated for each variable. Stepping method criteria were defined as .05 entry, .10 removel. Additionally, R square, ANOVA F values and Durbin-Watson values for linear regression are also provided. "Gender", "Mother educational attainment", "Father educational attainment", "Family structure", "Family income", "Have you ever lived with an elderly family member", "Would you prefer to live with your parents in the future ", "Willingness to work in institutions providing elder care services in the future" variables were included in the multiple linear regression analysis. Age was not included in the model because the ages of the participants were very close to each other. In order to include categorical variables in the multiple linear regression analysis, a reference variable was defined and dummy variables were created. For this purpose, the reference

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categories were defined as "0". All statistical analyses were conducted using STATA software (v.18, College Station, TX, USA), and the threshold value of statistical significance in all analyses was quantified at a two-tailed p-value of < 0.05.

Results

The sociodemographic and baseline characteristics of the research group, consisting a total of 158 participants, are summarized in Table 1.

Table 1. Sociodemographic and baseline characteristics of the research group (n = 158)

Variables	Total (n= 158)
Age, years, mean ± SD	23.9 ± 1.7
Mother age, years, mean ± SD	53.1 ± 5.3
Father age, years, mean ± SD	57.3 ± 6.1
Gender, n (%)	
Male	89 (56.3)
Female	69 (43.7)
Living arrangement, n (%)	
Family house	19 (12.0)
Student house	132 (83.5)
Dormitory	7 (4.4)
Mother educational attainment, n (%)	
High school and below	80 (50.6)
University and above	78 (49.4)
Father educational attainment, n (%)	
High school and below	72 (45.6)
University and above	86 (54.4)
Family structure, n (%)	
Nuclear	140 (88.6)
Extended	14 (8.9)
Single-parent	4 (2.5)
Family income, n (%)	
Lower than expenses	20 (12.7)
Equal to expenses	78 (49.4)
Higher than expenses	60 (38.0)
Have you ever lived with an elderly family member? n (%)	
Yes	85 (53.8)
No	73 (46.2)
Would you prefer to live with your parents in the future?	
Yes	46 (29.1)
No	112 (70.9)
Willingness to work in institutions providing elder care services in the future	
Yes	34 (21.5)
No	124 (78.5)

SD standard deviation

The mean age of the participants was 23.9 years (SD ± 1.7), while the mean ages of their mothers and fathers were 53.1 (SD ± 5.3) and 57.3 (SD ± 6.1) years, respectively. In terms of gender distribution, 56.3% of the participants were male (n = 89), and 43.7% were female (n = 69). Regarding living arrangements, the majority of participants (83.5%, n = 132) resided in student housing, while smaller proportions lived in family homes (12.0%, n = 19) or dormitories (4.4%, n = 7).

Parental educational attainment revealed that 50.6% of mothers had education levels of high school or below, while

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49.4% had university-level education or higher. Fathers demonstrated slightly higher educational attainment, with 54.4% having university degrees or above compared to 45.6% with high school education or below. Most participants came from nuclear families (88.6%, n = 140), followed by extended families (8.9%, n = 14) and single-parent households (2.5%, n = 4).

In terms of family income, nearly half of the participants (49.4%, n = 78) reported family incomes equal to their expenses, while 38.0% (n = 60) had incomes exceeding their expenses, and 12.7% (n = 20) had incomes lower than their expenses. Additionally, 53.8% of participants (n = 85) reported having lived with an elderly family member, while 46.2% (n = 73) had not. Lastly, when asked about future living preferences, 70.9% (n = 112) of participants expressed that they would not prefer to live with their parents in the future, while 29.1% (n = 46) indicated a preference to do so. These findings provide valuable insights into the sociodemographic profiles and familial dynamics of the study population.

The total scores and sub-scale scores of the UCLA Geriatrics Attitudes (UCLA-GA) Scale, which evaluates participants' attitudes toward elderly across four dimensions, are summarized in Table 2.

The Social Value sub-scale, consisting of two items, yielded a mean score of 6.2 ± 1.4, with possible scores ranging from 3 to 10, indicating moderate levels of perceived social value attributed to elderly. The Medical Care sub-scale, which includes four items, had a mean score of 14.4 ± 2.2, with a score range of 9 to 20, reflecting positive attitudes toward the medical care provided to elderly individuals. Similarly, the Compassion sub-scale, also comprising four items, demonstrated a mean score of 14.4 ± 2.3, with scores spanning from 4 to 20. The Resource Distribution sub-scale, with 4 items, had a mean score of 12.4 ± 2.1, ranging from 7 to 19, indicating moderate perceptions of fairness in resource allocation for elderly care. The overall UCLA-GA Scale total score, derived from all 14 items, averaged 47.5 ± 4.6, with a range of 36 to 58, suggesting generally positive attitudes toward elderly among the participants.

Table 2. Total and sub-scale scores of UCLA Geriatrics Attitudes (UCLA-GA) Scale of the participants

Sub-scale of attitudes scale	Number of question	Scores	Minumum score	Maximum score
Social value (SV)	2	6.2 ± 1.4	3	10
Medical care (MC)	4	14.4 ± 2.2	9	20
Compassion (CP)	4	14.4 ± 2.3	4	20
Resource Distribution (RD)	4	12.4 ± 2.1	7	19
Total	14	47.5 ± 4.6	36	58

The UCLA-GA Scale consists of 14 items, each rated on a five-point Likert scale from 1 (strongly disagree) to 5 (strongly agree). Consequently, the lowest possible total score on the scale is 14, while the highest possible score is 70.

Table 3. Distribution of participants' UCLA Geriatrics Attitudes Scale (UCLA-GA) total and subscale scores according to various variables

Variables	Social value	Medical care	Compassion	Resource Distribution	Total
Gender					
Female (n = 69)	5.9 ± 1.4	14.5 ± 2.2	14.6 ± 2.1	12.2 ± 2.2	47.4 ± 4.5
Male (n = 89)	6.4 ± 1.4	14.3 ± 2.2	14.2 ± 2.5	12.6 ± 2.1	47.6 ± 4.8
*P-value**	**0.044†**	0.493	0.323	0.331	0.801

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Living arrangement					
Family house (n = 19)	5.6 ± 1.4	14.7 ± 1.4	14.2 ± 3.0	12.0 ± 2.1	46.6 ± 4.6
Student house or Dormitory (n = 139)	6.3 ± 1.4	14.3 ± 2.3	14.4 ± 2.3	12.5 ± 2.1	47.6 ± 4.7
*P-value**	**0.049†**	0.501	0.755	0.322	0.361
Mother educational attainment					
High school and below (n = 80)	6.2 ± 1.4	14.4 ± 2.2	14.8 ± 1.9	12.4 ± 2.2	48.0 ± 4.8
University and above (n = 78)	6.2 ± 1.5	14.3 ± 2.2	13.9 ± 2.6	12.5 ± 2.1	47.0 ± 4.5
*P-value**	0.978	0.719	**0.016†**	0.802	0.206
Father educational attainment					
High school and below (n = 72)	6.2 ± 1.3	14.4 ± 2.0	14.9 ± 1.8	12.3 ± 2.3	47.9 ± 4.6
University and above (n = 86)	6.2 ± 1.5	14.3 ± 2.3	14.0 ± 2.7	12.5 ± 2.1	47.2 ± 4.7
*P-value**	0.849	0.866	**0.017†**	0.526	0.311
Family structure					
Nuclear (n = 140)	6.2 ± 1.4	14.5 ± 2.1	14.3 ± 2.3	12.5 ± 2.1	47.6 ± 4.7
Extended (n = 14)	5.8 ± 1.3	13.5 ± 2.4	15.4 ± 1.8	12.2 ± 2.1	47.1 ± 4.7
*P-value**	0.304	0.128	0.096	0.709	0.706
Family income, n (%)					
Lower than expenses (n = 20)	6.0 ± 1.5	14.9 ± 1.6	13.8 ± 2.1	12.6 ± 2.3	47.4 ± 4.8
Equal to expenses (n = 78)	6.2 ± 1.3	14.2 ± 2.3	14.6 ± 2.1	12.4 ± 2.4	47.7 ± 4.5
Higher than expenses (n = 60)	6.3 ± 1.5	14.4 ± 2.3	14.2 ± 2.6	12.4 ± 1.8	47.4 ± 4.8
*P-value***	0.715	0.482	0.303	0.919	0.926
Have you ever lived with an elderly family member?					
Yes (n = 85)	6.3 ± 1.3	14.2 ± 2.2	14.6 ± 2.2	12.0 ± 2.1	47.3 ± 4.5
No (n = 73)	6.1 ± 1.6	14.6 ± 2.2	14.1 ± 2.5	12.9 ± 2.1	47.7 ± 4.8
*P-value**	0.420	0.288	0.125	**0.016†**	0.557
Would you prefer to live with your parents in the future?					
Yes (n = 46)	6.1 ± 1.6	13.7 ± 2.4	14.2 ± 2.5	12.1 ± 2.0	46.2 ± 4.7
No (n = 112)	6.3 ± 1.3	14.6 ± 2.0	14.4 ± 2.3	12.6 ± 2.2	48.0 ± 4.5
*P-value**	0.505	**0.019†**	0.635	0.187	**0.028**
Willingness to work in institutions providing elder care services in the future					
Yes (n = 34)	6.0 ± 1.2	13.5 ± 1.8	15.1 ± 2.1	11.5 ± 2.2	46.3 ± 4.6
No (n = 124)	6.3 ± 1.5	14.6 ± 2.2	14.2 ± 2.4	12.7 ± 2.1	47.8 ± 4.6
*P-value**	0.336	**0.012†**	**0.038†**	**0.006†**	0.092

The UCLA-GA Scale consists of 14 items, each rated on a five-point Likert scale from 1 (strongly disagree) to 5 (strongly agree). Consequently, the lowest possible total score on the scale is 14, while the highest possible score is 70. * Independent sample t-test (two-tailed p value). **ANOVA (One-way). † Statistically significant.

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The responses to the question "What comes to mind when you think of aging?" reveal a variety of perspectives, with recurring themes related to the physical, emotional, and social aspects of aging. The most frequently mentioned associations include disease (11.4%), retirement (7.0%), and death (6.3%). Many participants highlighted physical changes such as wrinkles, graying hair, and a decline in body functionality, emphasizing the physical toll of aging. Emotional responses included feelings of loneliness, helplessness, and fear, while social aspects often revolved around dependence on others, family dynamics, and reduced mobility. Positive associations were rare but included terms like maturity, experience, and peaceful time with family. Overall, the data suggest that aging is predominantly viewed through a lens of challenges, particularly those related to health, independence, and vitality.

The responses to the question "Would you like to work in an institution providing elderly care in the future?" reveal a predominantly negative sentiment among intern doctors. A significant proportion of participants (11.4%) cited the complexity of care for elderly patients, including managing comorbidities and the physical and emotional challenges associated with elderly. Emotional difficulty, such as the burden of witnessing frailty, illness, and mortality, was mentioned by 6.3% of respondents. Similarly, 7.0% expressed that working with elderly individuals does not align with their career goals, preferring fields like pediatrics, surgery, or more dynamic environments. Furthermore, communication challenges with elderly patients were emphasized by 1.9% of participants, and 1.3% specifically highlighted the emotional toll of constant exposure to aging and mortality. Overall, these findings indicate that most intern doctors feel unmotivated or unsuited for careers in elderly care due to professional misalignment, emotional burden, and the perceived complexity of managing this patient population.

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The UCLA Geriatrics Attitudes Scale scores and subscales were analyzed across various participant characteristics, yielding significant insights in Table 3. Male participants exhibited significantly higher scores on the Social Value subscale compared to females (6.4 ± 1.4 vs. 5.9 ± 1.4, $p = 0.044$). Participants residing in student housing or dormitories scored higher on the Social Value subscale than those living in family homes (6.3 ± 1.4 vs. 5.6 ± 1.4, $p = 0.049$). Parental educational attainment also influenced attitudes; participants with mothers ($p = 0.016$) or fathers ($p = 0.017$) who attained university-level education scored significantly higher on the Compassion subscale than those whose parents had high school education or below. Participants who had not lived with elderly family members scored significantly higher on the Resource Distribution subscale compared to those who had (12.9 ± 2.1 vs. 12.0 ± 2.1, $p = 0.016$). Furthermore, individuals who expressed a preference not to live with their parents in the future scored higher on the Medical Care subscale (14.6 ± 2.0 vs. 13.7 ± 2.4, $p = 0.019$) and the total UCLA-GA score (48.0 ± 4.5 vs. 46.2 ± 4.7, $p = 0.028$). Lastly, participants unwilling to work in elderly care institutions demonstrated significantly higher scores on the Medical Care (14.6 ± 2.2 vs. 13.5 ± 1.8, $p = 0.012$), Compassion (15.1 ± 2.1 vs. 14.2 ± 2.4, $p = 0.038$), and Resource Distribution (12.7 ± 2.1 vs. 11.5 ± 2.2, $p = 0.006$) subscales.

The multiple linear regression analysis summarized in Table 4 examines the predictors influencing the total and sub-scale scores of the UCLA-GA.

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Table 4. Multiple linear regression analysis examining the effects of relative variables on changes in total and sub-scale of UCLA-GA scores

Predictor	Unstandardized coefficients B	95% CI for B	Coefficients Std. Error	Standardized coefficients B	*P*-value
Total Score[a]					
Would you prefer to live with your parents in the future?	1.793	0.198–3.388	.807	.174	**0.028**†
Willingness to work in institutions providing elder care services in the future	1.527	-.236–3.291	.893	.134	0.089
R square, ANOVA (F), Durbin-Watson, *p*-value	0.048	3.942	2.106		**0.021**†
Social value scores[b]					
Gender	.475	.012–.938	.234	.160	**0.044**†
R square, ANOVA (F), Durbin-Watson, *p*-value	.026	4.110	1.907		**0.044**†
Medical care scores[c]					
Family structure	-1.031	-2.097 – .034	.539	-.147	0.058
Would you prefer to live with your parents in the future?	.954	.208 – 1.699	.377	.194	**0.012**†
Willingness to work in institutions providing elder care services in the future	1.078	.255 – 1.900	.416	.198	**0.011**†
R square, ANOVA (F), Durbin-Watson, *p*-value	.096	5.451	1.825		**0.001**†
Compassion scores[d]					
Mother educational attainment	-1.175	-1.921 – -.428	.378	-.247	**0.002**†
Family income	.970	-.144 – 2.085	.564	.136	0.088
Willingness to work in institutions providing elder care services in the future	-1.097	-1.984 – -.210	.449	-.189	**0.016**†
R square, ANOVA (F), Durbin-Watson, *p*-value	.091	5.151	2.059		**0.002**†
Resource Distribution scores[e]					
Have you ever lived with an elderly family member?	.736	.063 – 1.408	.341	.168	**0.032**†
Willingness to work in institutions providing elder care services in the future	1.057	.241 – 1.873	.413	.199	**0.011**†
R square, ANOVA (F), Durbin-Watson, *p*-value	.075	6.321	2.255		**0.002**†

** Multiple linear regression included variables with $p < 0.100$ criterion and calculated by using the backward stepwise method. Reference for gender is "female"; Reference for Would you prefer to live with your parents in the future? is "yes"; Reference for Willingness to work in institutions providing elder care services in the future is "yes"; Reference for Family structure is "nuclear family"; Reference for Have you ever lived with an elderly family member? is "yes"; Reference for Mother educational attainment is "High school and below". † Statistically significant.a Seven steps. b Eight steps. c Six steps. d Six steps. e Seven steps.

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For the Total Score, a significant positive association was observed with the preference to live with parents in the future (β = 1.793, 95% CI: 0.198–3.388, p = 0.028). This finding suggests that intern doctors who prefer living with their parents have a more favorable overall attitude toward older adults. However, willingness to work in institutions providing elder care services showed a positive trend without statistical significance (p = 0.089). n the Social Value subscale, gender emerged as a significant predictor (β = 0.475, 95% CI: 0.012–0.938, p = 0.044), with males showing higher scores compared to females. This indicates that male participants hold stronger positive views regarding the societal contributions of older individuals. For the Medical Care subscale, both the preference to live with parents in the future (β = 0.954, 95% CI: 0.208–1.699, p = 0.012) and willingness to work in elder care institutions (β = 1.078, 95% CI: 0.255–1.900, p = 0.011) were significantly associated with higher scores. In the Compassion subscale, maternal educational attainment (β = -1.175, 95% CI: -1.921 to -0.428, p = 0.002) and willingness to work in elder care institutions (β = -1.097, 95% CI: -1.984 to -0.210, p = 0.016) were significant. Lower maternal educational levels were associated with higher compassion scores, highlighting the complex interplay of socioeconomic and educational factors in shaping empathetic attitudes. Finally, for the Resource Distribution subscale, participants who had lived with an elderly family member (β = 0.736, 95% CI: 0.063–1.408, p = 0.032) and those willing to work in elder care institutions (β = 1.057, 95% CI: 0.241–1.873, p = 0.011) had significantly higher scores. This suggests that direct experience with older adults positively influences perceptions of fairness in resource allocation for elderly care.

Discussion

This study provides valuable insights into the attitudes of intern doctors toward elderly, assessed through the UCLA-GA Scale. The findings highlight the influence of demographic factors, personal experiences, and professional aspirations on attitudes toward elderly care. The results indicate generally positive attitudes toward g elderly among participants, consistent with prior studies using the UCLA-GA scale. For instance, research conducted by Şahin et al. (2012) demonstrated favorable attitudes toward elderly patients among healthcare providers, underscoring the reliability of the scale as a measurement tool (18). Similarly, Al Ghailani et al. (2024) reported moderate to positive attitudes toward elderly among medical students and doctors, emphasizing the need for improved elderly education in medical curricula to further enhance these perspectives (19). Additionally, a study by De Biasio et al. (2016) highlighted that while medical students exhibit initially positive attitudes, their perceptions may decline without ongoing curriculum-based interventions, indicating the importance of sustained engagement with geriatrics throughout training (20). This study also found that factors such as parental education and previous living experiences with elderly family members significantly shaped participants' perspectives, reinforcing the role of familial and environmental contexts in the development of empathy and compassion.

The participants predominantly associated aging with negative themes such as illness, dependency, and death, reflecting broader societal stereotypes. These findings align with prior literature highlighting ageism as a pervasive influence on medical professionals' perceptions of aging (21). While some participants recognized positive aspects of aging, such as wisdom and family bonding, these responses were comparatively rare, emphasizing the need for targeted educational interventions to foster a more balanced understanding of aging. Of note, a significant proportion of participants expressed reluctance to pursue careers in geriatrics, citing factors such as the emotional burden of witnessing frailty and mortality, the complexity of managing comorbidities, and a perceived misalignment with career aspirations. These findings echo prior research suggesting that while medical students may hold positive attitudes toward elderly care, they often view geriatrics as a less desirable specialty (20). Interventions such as mentorship programs and enhanced exposure to geriatric medicine during training may mitigate these perceptions.

Male participants and those living in student housing exhibited higher scores on the Social Value subscale, suggesting that peer-based and collaborative living environments may positively influence perceptions of elderly. Inversely, in a related Turkish study conducted at Necmettin Erbakan University, gender differences and income levels significantly influenced attitudes, with female students and those with higher incomes demonstrating more positive perceptions (22). This finding aligns with studies indicating that social contexts can shape attitudes toward elderly care (23). Similarly, Al Ghailani et al. (2024) highlighted that living arrangements and personal exposure to elderly individuals play a significant role in shaping positive attitudes among medical students (19). Additionally, a study by De Biasio et al. (2016) noted that structured educational and social exposures during medical training could help foster a better

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understanding of the social value of geriatrics (24). These findings collectively underscore the importance of social and environmental factors in shaping perceptions of elderly care.

Participants whose parents attained higher education levels demonstrated greater compassion scores. This observation suggests that early familial influences and socioeconomic factors play critical roles in shaping empathy toward elderly individuals. These results align with studies emphasizing the importance of personal background in shaping attitudes among healthcare trainees (19). Similarly, Zanjari et al. (2022) reported that familial socioeconomic status and parental education significantly impacted healthcare professionals' attitudes toward geriatrics, further reinforcing the role of early influences (21). Additionally, a study by De Biasio et al. (2016) highlighted that students from higher socioeconomic backgrounds tend to exhibit more empathetic attitudes, suggesting that educational interventions targeting empathy could help mitigate disparities (24).

Of note, participants unwilling to work in geriatrics scored higher on the Medical Care and Compassion subscales. This may reflect a heightened awareness of the demands of elderly care, leading to self-selection away from the specialty. Similar findings have been noted in previous studies, where increased awareness of challenges in elderly care correlated with hesitancy to specialize in geriatrics (25). Additionally, Chua et al. (2008) observed that while medical students demonstrated positive attitudes toward elderly care, many cited the emotional and logistical challenges of geriatrics as reasons for reluctance to pursue it as a career (23). Moreover, De Biasio et al. (2016) emphasized that structured exposure to geriatrics during medical training could help address misconceptions, although the inherent complexity of the field remains a deterrent for many trainees (24). These findings suggest that hesitancy to specialize in geriatrics may arise not from a lack of empathy but from an informed understanding of the specialty's challenges.

The findings of this study highlight the need for curriculum reforms to address stereotypes and foster positive attitudes toward elderly. Integrating empathy-building modules, early clinical exposure to elderly care, and structured mentorship programs can help address the challenges identified in this study. Evidence suggests that holistic educational interventions can improve both knowledge and attitudes toward elderly (26). For example, Goeldlin et al. (2014) demonstrated that geriatric clinical skills training modestly improved attitudes, particularly in the domain of resource distribution (25). Similarly, Haque et al. (2013) found that geriatric-focused workshops helped medical students maintain positive attitudes toward elderly care, even when baseline interest in geriatrics was low (27). Additionally, another study by Çalışkan et al. (2018) stated that it is necessary to include more elderly content in the training curriculum in the pre-graduation education to improve the attitudes of family physicians towards elderly care (22). These findings reinforce the importance of embedding targeted, empathy-building educational strategies into medical training to combat stereotypes and improve perceptions of elderly care.

The study has several limitations that should be acknowledged. First, the sample was drawn from a single institution, which may limit the generalizability of the findings to other medical schools or regions with different sociocultural contexts. Additionally, the cross-sectional design provides only a snapshot of attitudes at a single point in time, making it difficult to capture longitudinal changes in perceptions and the impact of ongoing training. The reliance on self-reported data through tools like the UCLA-GA scales introduces the potential for response bias, as participants may have provided socially desirable answers rather than accurate reflections of their true attitudes. The study also faced a notable limitation in the relatively high proportion of individuals who chose not to participate, which may have introduced selection bias. Those who opt out might have had different attitudes or experiences regarding elderly, potentially influencing the overall results and limiting the representativeness of the findings. This non-participation could have skewed the data toward those with inherently more interest or positive attitudes toward elderly care, thus warranting caution in interpreting the outcomes. Another limitation is the short-term evaluation of the intervention, as the study did not explore the long-term effects of screening on attitudes. The study also lacked a detailed analysis of the medical curriculum and the specific elderly training provided, which could have contextualized the findings more effectively. Finally, while validated tools were used, they may not fully capture the complexity of attitudes toward elderly; incorporating qualitative methods such as interviews or focus groups could have provided a deeper understanding of the results. These limitations underscore the need for broader, longitudinal studies and more comprehensive evaluations of educational strategies in geriatrics.

Conclusions

Taken together, the outcomes of this study highlight the multifaceted factors influencing medical trainees' attitudes toward the elderly, including personal demographics, cultural influences, and exposure to elderly care. Personal and professional inclinations significantly shape these attitudes. These factors not only shape perceptions of aging but also

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influence career preferences and willingness to specialize in geriatrics. Those who preferred to live with their parents in the future exhibited more favorable perceptions of aging, while willingness to work in elder care institutions was associated with higher scores of medical care and resource distribution subscales. Despite these positive perspectives, aging remains largely perceived through negative lenses, such as illness, dependency, and mortality, emphasizing the need for targeted educational interventions to foster a more balanced and nuanced understanding of aging among future healthcare professionals. Addressing these challenges through targeted educational strategies such as empathy-building workshops, early clinical exposure, and structured mentorship programs is essential for fostering positive attitudes. Additionally, incorporating arts-based interventions, such as films or role-play, can help trainees better understand the emotional and social dimensions of aging, though these methods should be carefully adapted to the cultural and personal contexts of the learners. Mentorship opportunities with experienced geriatricians can also inspire students and mitigate concerns about the perceived challenges of the field. These targeted interventions are critical for preparing future healthcare professionals to meet the demands of an aging population, ensuring they possess the knowledge, skills, and attitudes necessary to deliver high-quality care.

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Investigation of the epigenetic response to the apoptotic effect of caspase-3 in colon cancer cell line (HT-29)

Kolon kanseri hücre hattında (HT-29) kaspaz-3'ün apoptotik etkisine epigenetik yanıtın incelenmesi

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Epigenetic response to the apoptotic effect of caspase-3
Received: 19.02.2025
Accepted: 12.06.2025
Doi:10.18614/dehm.1751132

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BACKGROUND
Colorectal cancer represents the third most prevalent cause of cancer-related fatalities, attributable to its high rate of recurrence and mounting resistance to existing therapeutic interventions. The objective of this study was to undertake a comparative analysis of caspase-3, HDAC1 and HDAC2 gene expression levels in HUVEC and HT-29 cells, with a view to elucidating the interplay between apoptotic and epigenetic mechanisms.

METHODS
The study was conducted by culturing the HT-29 and HUVEC cell lines under appropriate conditions and performing real-time PCR to measure the gene expression levels of caspase-3, HDAC1, and HDAC2 in the cells.

RESULTS
The results obtained revealed a high level of caspase-3 activity and a low levels of HDAC1 and HDAC2 activity in the HUVEC cells.In contrast, a low level of caspase-3 activity and high levels of HDAC1 and HDAC2 activity were detected in the HT-29 cells.

CONCLUSION
In conclusion, the present study demonstrated that the epigenetic response to the apoptotic effect of caspase-3 in the colon cancer cell line (HT-29) and the HUVEC cell line was found to be associated with a negative correlation, suggesting that these genes may serve as potential biomarkers for colon cancer diagnosis and treatment.

KEYWORDS
Caspase-3, colon cancer, HT-29, HDAC1, HDAC2, HUVEC

ÖZ

AMAÇ
Kolorektal kanser, yüksek tekrarlama oranı ve mevcut tedavilere karşı artan direnç nedeniyle kanserle ilişkili ölümlerin üçüncü en yaygın nedenidir. Bu çalışmanın amacı, apoptotik ve epigenetik mekanizmalar arasındaki etkileşimi aydınlatmak amacıyla HUVEC ve HT-29 hücrelerinde kaspaz-3, HDAC1 ve HDAC2 gen ekspresyon seviyelerinin karşılaştırmalı bir analizini yapmaktır.

GEREÇ YÖNTEM
Çalışma, HT-29 ve HUVEC hücre hatlarının uygun koşullar altında kültüre edilmesi ve hücrelerdeki kaspaz-3, HDAC1 ve HDAC2 gen ekspresyon seviyelerini ölçmek için gerçek zamanlı PCR yapılmasıyla gerçekleştirilmiştir.

BULGULAR
HUVEC hücrelerinde yüksek kaspaz-3 aktivitesi, düşük HDAC1 ve HDAC2 aktivitesi bulunmuşken, HT-29 hücrelerinde düşük kaspaz-3 aktivitesi, yüksek HDAC1 ve HDAC2 aktivitesi bulunmuştur.

SONUÇ
Sonuç olarak bu çalışma kolon kanseri hücre hattında (HT-29) ve HUVEC hücre hattında kaspaz-3'ün apoptotik etkisine verilen epigenetik yanıtın negatif korelasyonla ilişkili olduğunu göstermiştir ve bu genlerin kolon kanseri tanı ve tedavisi için potansiyel biyobelirteçler olarak hizmet edebileceğini düşündürmektedir

ANAHTAR KELİMELER
HT-29, HDAC1, HDAC2, HUVEC, kaspaz-3, kolon kanseri.

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Epigenetics is defined as alterations in gene expression that are capable of being inherited both mitotically and/or meiotically, and which do not necessitate a change in the DNA sequence (1). In cancerous cells, there is frequently an occurrence of aberrant epigenetic and gene expression patterns, consequent to the perturbation of oncogenes and tumour suppressor genes. The potential for the reversal of epigenetic modifications renders the reprogramming of cancer cells through epigenetics, which is important in preventing, controlling, and treating cancers (2).

Histones and DNA interactions regulate the process of gene transcription, whereby the process of transcription is initiated or suppressed. There are several chemical modifications, particularly acetylation of lysine residues, which can alter the position of histones and affect gene transcription. Since excessive deacetylation of histones has been associated with the pathology of cancer diseases, it can be concluded that this process promotes the repression of tumour regulatory genes (3). Histone acetylation is a process that is subject to regulation by two enzymes: histone acetyltransferases (HATs) and histone deacetylases (HDACs).

The HDACs referred to above can be categorised into four distinct classes: class I encompasses HDACs 1, 2, 3 and 8; class II comprises HDACs 4, 5, 6, 7, 9 and 10; class III consists of sirtuins (SIRT1-7); and class IV consists of HDAC 11, which exhibits characteristics of both class I and II HDACs. The delicate balance between histone acetylation and deacetylation is critical for the regulation of gene expression, and aberrant expression and mutations of genes encoding HDACs have been linked to various disorders, including those affecting critical cellular functions such as cell proliferation, cell cycle regulation and apoptosis, and, consequently, tumour development (4). In a variety of cancerous cell types, including colorectal cancer cells, histone deacetylase (HDAC) inhibitors have been shown to induce cell cycle arrest, cell differentiation and apoptosis, as well as decreasing metastasis (3,5). A significant number of studies have demonstrated that class I HDACs are overexpressed in cancerous cells and are particularly involved in the differentiation of colorectal cancer cells (6).

The Caspase family constitutes a cysteine protease family which exerts pivotal functions in the domains of programmed cell death and inflammation. (7). Increasing evidence reveals that caspase-3 has critical functions beyond apoptosis, performing pro-survival functions in malignant transformation and tumorigenesis. However, the mechanism of the non-apoptotic effect of caspase-3 in oncogenic transformation has remained unclear (7, 8).

In the present study, the HT-29 and HUVEC cell lines were examined. The purpose of the comparison of HT-29 and HUVEC cells is due to their widespread use in cancer research. These cell lines are of significant value in the context of understanding the growth, spread, and response of cancer cells to therapy.

The objective of the present study was to make a comparison between the levels of gene expression of Caspase-3, HDAC1, and HDAC2 in HUVEC and HT-29 cells, and to ascertain the association between apoptotic and epigenetic mechanisms. The extant literature does not yet include studies that compare the expressions of caspase-3 and HDAC1/2 in HUVEC cells and other cancer cells, including colon cancer. A review of extant literature reveals that studies in this field have been conducted by applying HDAC inhibitors or drug therapy. The present study provides novel information to the existing literature by addressing this gap in knowledge.

Materials and Methods

Cell culture

The human colon cancer cell lines (ATCC: HTB-38) and human umbilical vein endothelial cell lines (ATCC: CRL-1730) were opened under appropriate conditions and transferred into DMEM medium containing 20% foetal bovine serum (FBS), 1% penicillin-streptomycin, and 1% L-glutamine. The cells were then cultured in a 5% CO2 incubator at 37°C until a sufficient number of cells were harvested by changing the medium containing 10% FBS, 1% Pen-Strep, and 1% L-Glutamine when necessary.

RNA extraction and cDNA synthesis

The cells were seeded in 6-well plates at a density of 6x104 cells per well, with three replicates being used for each condition. Following a period of incubation, the cells were collected and subjected to a centrifugation process at 4000 rpm for 10 minutes at 4°C. Subsequent to this, the cells were transferred to 1,5 mL eppendorf tubes, and RNA isolation was performed. The RNA isolation process was conducted using

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the TRIzol reagent (RiboEx Kit; GeneAll Biotechnology, Seoul, Korea), following the manufacturer's instructions. The trisol step was utilised as the inaugural phase, designated as the lysis phase, with a view to enhancing the efficiency of the isolation process. Subsequently, The RNA isolation kit (GeneAll Biotechnology, Hybrid-R, Seoul, Korea), which is based on the spin column technique, was proceeded. The concentration and purity of the RNA were subsequently measured using the Nanodrop device Quibit (Invitrogen, Qubit4 Fluorometer). In this experiment, ABT, cDNA Synthesis Kit (Rnase Inh. High Capacity, Türkiye), was utilised for the synthesis of cDNA. The master mix was prepared in a total volume of 20 µl, and reverse transcription was performed. The processing steps were analyzed in the ProFlex thermal cycler at 25°C for 10 min, 37°C for 120 min, and 85°C for 5 min (1 cycle). The cDNA samples synthesized were stored at -80°C.

Quantitative real time PCR

For the real-time PCR analysis, the ABT 2X SYBR Mastermix kit (Türkiye) was utilised, with the master mix being prepared in a total volume of 20 µL in accordance with the manufacturer's instructions. The reaction was then performed on an Applied Biosystems QuantStudio5 Real-Time PCR instrument. The thermal cycling parameters for the PCR reaction included an initial denaturation step at 95°C for 300 seconds, followed by 40 cycles of denaturation at 95°C for 15 seconds and an annealing step at 60°C for 60 seconds. The samples were analysed in triplicate, and the specific primers utilised are listed in Table 1. Primers were specifically designed utilising primer3plus and the NCBI database. The designed primers have been validated using in silico primer analysis tools.

Table 1. Primers used in real time-qPCR

Primer name	Primer sequence 5′-3′
HS-GAPDH-F	AGGGCTGCTTTTAACTCTGGT
HS-GAPDH-R	CCCCACTTGATTTTGGAGGGA
HS-HDAC2-F	TTACTGATGCTTGGAGGAGGT
HS-HDAC2-R	TTCTGGAGTGTTCTGGTTTGT
HS-HDAC1-F	CCTGGAACTGCTAAAGTATCACC
HS-HDAC1-R	ACTCGTCATCAATCCCGTCT
HS-CASP3-F	ATTTGGAACCAAAGATCATACATGG
HS-CASP3-R	TTCCCTGAGGTTTGCTGCAT

Statistical analysis

All data was analyzed using IBM SPSS 25.0 (Chicago, USA) and GraphPad Prism (Version 8.02, USA). The data were determined to be normally distributed (Shapiro-Wilk test, $P>0.05$) and homogeneous (Levene's test). The data were presented as mean ± standard deviation (SD) of at least three independent experiments. Student T-test was used for statistical analyses of gene expressions between the two groups. In addition, the Tukey post-hoc test and two-way analysis of variance (ANOVA) test were used to compare gene expressions between HUVEC and HT29 cell lines. $p < 0.05$ was considered to be statistically significant.

Ethical statement and Informed consent

The present study does not include an ethical statement on account of the fact that its focus is a cell culture study. The requirement for informed consent was not applicable to this study due to the utilisation of cell lines.

Results

The present study set out to investigate the expression levels of the caspase-3, HDAC1, and HDAC2 genes in two different cell lines. The analysis was conducted using the HT29 and HUVEC cell lines. The gene expression levels of the abovementioned genes were then compared in these two groups of cells. The Student T-test results used in the statistical evaluation of gene expressions between the two groups are demonstrated in Table 2. The statistical analysis revealed that Caspase-3, HDAC1, and HDAC2 gene expression levels significantly differed between the HT29 and HUVEC cell lines ($p < 0.05$).

The analysis revealed a statistically significant decrease in the expression of the caspase-3 gene in the HT29 cell line compared to the HUVEC cell line. In addition, the analysis revealed a significant increase in the expression of the HDAC1 and HDAC2 genes in the HT29 cell line. The results of the study are presented in Figure 1.

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Table 2. Student T test results between the groups (F: F value, Sig: Significant, DF: Degree of Freedom)
Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
CASP3	Equal variances assumed	2.539	.186	3.506	4	.025	1.32333	.37748	.27529	2.37138
	Equal variances not assumed			3.506	2.841	.043	1.32333	.37748	.08313	2.56353
HDAC1	Equal variances assumed	2.551	.185	-4.533	4	.011	-1.10333	.24340	-1.77913	-.42754
	Equal variances not assumed			-4.533	2.594	.027	-1.10333	.24340	-1.95131	-.25536
HDAC2	Equal variances assumed	1.046	.364	-5.709	4	.005	-1.12000	.19619	-1.66470	-.57530
	Equal variances not assumed			-5.709	3.464	.007	-1.12000	.19619	-1.69957	-.54043

Figure 2 demonstrates a differential expression between each other of the Caspase-3, HDAC1, and HDAC2 gene expressions observed between the HUVEC and HT29 cell lines. In the HUVEC cell line, the expression level of the caspase-3 gene was determined to be the highest in comparison to the expression levels of the HDAC1 and HDAC2 genes. A statistically significant difference was detected ($p < 0.05$). A negative correlation was observed between the caspase-3/HDAC1 and caspase-3/HDAC2 genes, and it was noted that there was a statistically significant difference between them ($p < 0.05$). No statistically significant relationship was found between the expression levels of the HDAC1 and HDAC2 genes ($p > 0.05$). In the HT29 cell line, the expression level of the caspase-3 gene was determined to be the lowest in comparison to the expression levels of the HDAC1 and HDAC2 genes, and a statistically significant difference was identified between them ($p < 0.05$). A negative correlation was observed between the caspase-3/HDAC1 and caspase-3/HDAC2 genes and it was noted that there was a statistically significant difference between them ($p < 0.05$). However, no statistically significant difference was found between the expression levels of the HDAC1 and HDAC2 genes ($p > 0.05$).

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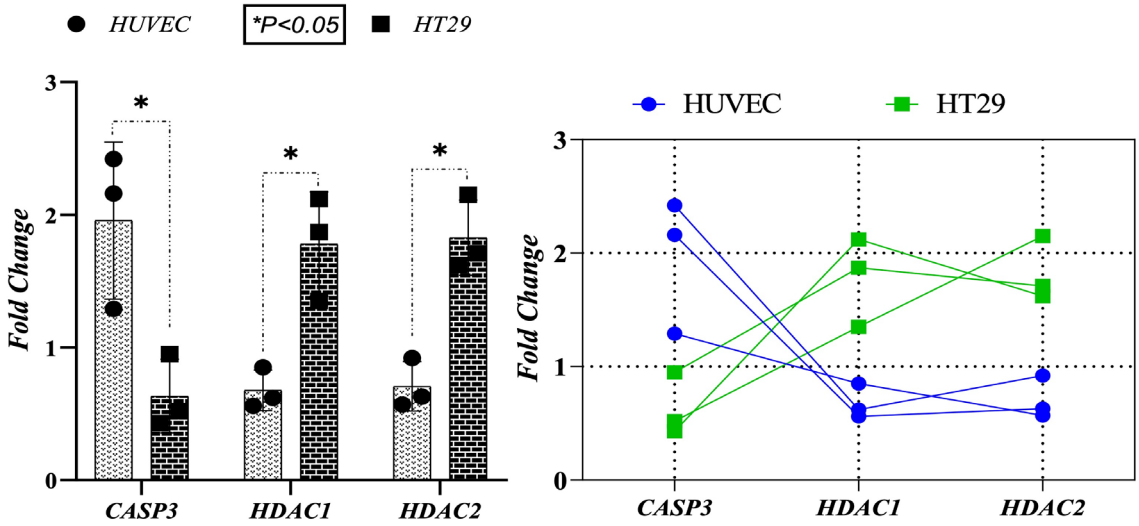

Figure 1. Caspase-3, HDAC1 and HDAC2 gene expression levels in HUVEC and HT29 cell lines. The results were presented as the mean ± standard deviation (SD) of three independent experiments (n = 3).

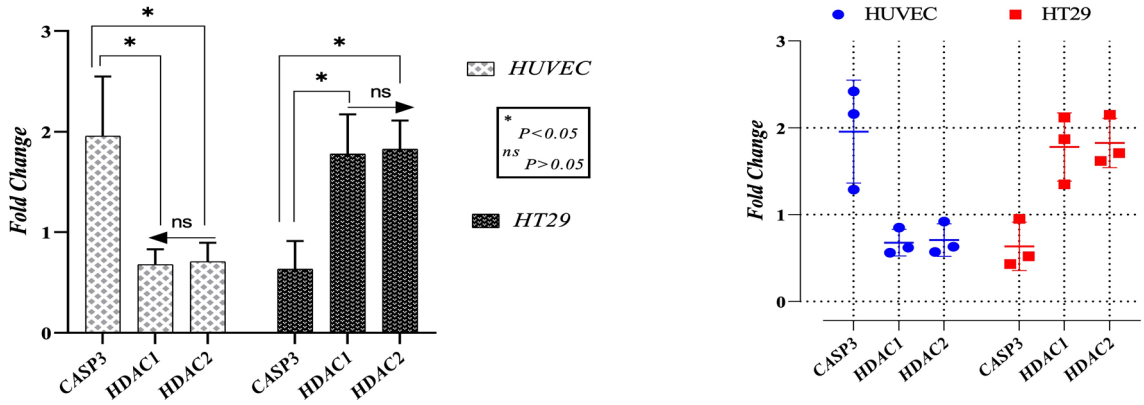

Figure 2. Evaluation between each other of Caspase-3, HDAC1 and HDAC2 gene expressions in the HUVEC and HT29 cell lines. The results were presented as the mean ± standard deviation (SD) of three independent experiments (n = 3).

Discussion

Carcinogenesis is a multi-stage process in which cells obtain various critical properties as a result of genetic instability and alterations in gene expression (9). While colorectal cancer is the second leading cause of cancer-related deaths, it is the fourth most frequently diagnosed cancer type worldwide (10). It has been established that HDACs are not functioning correctly in cases of cancer. Consequently, several HDAC inhibitors are currently being investigated for their potential use as cancer chemotherapeutics (11). A plethora of scientific studies have demonstrated that there is an increase in HDAC activity in cases of colon cancer (9, 12-15). It has been reported that HDAC inhibitors can induce apoptosis in various cancer cell lines, including those derived from breast cancer (MCF-7), lung cancer and colon cancer (HCT116) (16). HDAC has been shown to function as a strong negative regulator of apoptosis and autophagy in tumourigenesis. The process of

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permeabilisation of the HDAC inhibitor results in the negative regulation of cancer progression, achieved by the inactivation of cytosolic HDAC.

This, in turn, leads to the activation of both apoptotic and autophagic pathways. It has been established that HDAC inhibition promotes extrinsic apoptosis by activating caspase 8 through the cell death receptors FADD, TRADD, TRAIL, and TNF. Furthermore, it has been demonstrated that HDACs are capable of suppressing the expression of anti-apoptotic proteins, including Bcl-2, Bcl-XL, and Mcl-1. This suppression, in turn, has been observed to stimulate the expression of pro-apoptotic proteins, such as Bax, Bad, Bid, Bak, NOXA, and PUMA (17). Studies performed in HT-29 cells in different years, showed that the active form of caspase-3 increased 24 hours after HDAC inhibitors were applied (18,19). In a 2023 study, HDAC inhibitors were similarly applied to HCT-116 and HT-29 cells. The results demonstrated that the levels of cleaved forms of PARP, caspase 3, caspase 8, and caspase 9 increased in both cells 24 hours after application. It is evident that these findings are consistent with our own (20). In a further study, it was noted that activation of caspase-3 and poly (ADP-ribose) polymerase 1 (PARP1) increased in HCT116 colon cancer cells treated with 5-fluorouracil. Following the suppression of HDAC1, it was established that the enhanced activation of caspase-3 was markedly repressed in cells treated with 5-fluorouracil (21). In a study by Min et al., in MCF-7 breast cancer cells, for the increased HDAC activity, HDAC inhibitors were used, and it was emphasised that these inhibitors activated caspase-3/7 and may induce caspase-dependent apoptosis partially through the mitochondrial pathway accompanied by an increased rate of cytochrome C release (16). Many studies have reported that the level of HDAC1 expression increases in colorectal cancer tissue in comparison with normal tissue (22).

However, another study found no significant difference between colorectal cancer tissue and normal tissue (23). In a study by Cao et al., it was demonstrated that the expression of HDAC1 was elevated in colorectal cancer tissues in comparison with normal tissues (24). Similarly a study by Huang et al. showed that the expression level of HDAC1 was elevated in colorectal cancer samples in comparison to normal mucosa. Moreover, these findings were found to be significantly associated with the survival outcomes of patients diagnosed with colorectal cancer (25). A separate study likewise emphasised that HDAC1 increases the survival, proliferation and transformation of colorectal cancer cells (26). As demonstrated by Qi et al., HDAC2 expression is significantly elevated in colorectal cancer in comparison to adjacent normal mucosa. The study further elucidates that high HDAC2 expression is associated with reduced survival and liver metastasis, as well as with higher T-stages. Additionally, the study emphasises that the downregulation of HDAC2 impedes cell migration and invasion (27). In a further study, HDAC2 was observed to be significantly overexpressed in both adenoma and colorectal cancer (28). In the course of our study, we discovered that the expression levels of HDAC1 and HDAC2 were significantly elevated in the colon cancer cell line HT29 in comparison to the HUVEC cell line. This increase was found to be statistically significant ($p < 0.05$).

The data obtained in the present literature review to determine the effect of the apoptotic regulator caspase-3 on HT29 and HUVEC cells were in the form of a comparison of colon cancer and HUVEC cells by applying a medical agent. For instance, in a study conducted in 2018, colorectal cancer cells and HUVEC cells were exposed to titanium dioxide nanoparticles at different doses, and RT-PCR was used to evaluate the expression of P53, Bax, Bcl-2, and caspase-3. Titanium dioxide nanoparticles had no significant effect on HUVECs, but caused a significant increase in caspase-3 expression in HT29 cells (29). In another study, the induction of apoptosis with Gentiopicroside in HCT116 colon cancer cells via Bax/Bcl2 and caspase-3 was observed, and the study demonstrated that gentiopicroside exerts a cytotoxic effect on colorectal cancer cells (30). In a previous study on colon cancer, the emphasis was placed on the fact that oncogenic alterations rendered cancer cells resistant to apoptosis. It was thus hypothesised that the activation of alternative cell death pathways might provide new therapeutic options. In addition, the genetic loss of caspase-3 in colon cancer cells was shown to increase susceptibility to DNA-damaging agents through RIP1-dependent necrosis, without abandoning apoptosis (31). Flanagan et al. reported that patients suffering from colorectal cancer who exhibited reduced levels of activated caspase-3 experienced a greater duration of disease-free survival (32). Nevertheless, recent studies have presented a more intricate profile of caspase-3 in the context of cancer development and therapeutic interventions. These studies have suggested that

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contrary to its role as a tumour suppressor, caspase-3 promotes carcinogenesis following cellular exposure to chemicals and radiation (7, 33). A study was conducted to ascertain whether caspase-3 activity could serve as a prognostic biomarker for colorectal cancer. Enzyme activity was correlated with clinical parameters, and elevated caspase-3 activity in tumours was found to be significantly associated with an increased risk of distant recurrence (34). The results of our present study demonstrate that there is a statistically significant decrease in the expression level of caspase-3 in HT29 colon cancer cells in comparison with HUVEC cells and that there is a statistically significant difference between the HUVEC and HT29 cell lines ($p < 0.05$).

In the present study, owing to restrictions in financial resources, the number of repetitions, which is generally regarded as the minimum level for statistical analysis in scientific research and biological studies, was constrained to three. This study was conducted in a setting in vitro. While in vitro experiments offer valuable insights, they might not entirely replicate the intricate interactions observed in living organisms. Consequently, there is a necessity for further in vitro and in vivo studies to be conducted in this area.

In conclusion, the present study demonstrated that the epigenetic response to the apoptotic effect of caspase-3 in the colon cancer cell line (HT-29) and the HUVEC cell line was found to be associated with a negative correlation between the caspase-3/HDAC1 and caspase-3/HDAC2 genes, suggesting that these genes may serve as potential biomarkers for colon cancer diagnosis and treatment. The combination of therapies employing HDAC inhibitors in the treatment of cancer has the potential to result in a substantial increase in patient survival time. Furthermore, the elevated expression of HDACs and the diminished expression of caspase3 may serve as promising biomarker candidates for the early diagnosis of the condition and monitoring prognoses. A number of HDAC inhibitors are already in clinical use for the treatment of various types of cancer, including lymphoma.

Conflict of interest

The authors declare no conflict of interest.

The contributions of the authors

VG designed, conceived and performed the research. TK performed statistical analysis. VG and TK confirm the raw data's authenticity. All authors read and approved the final manuscript.

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Evaluation of medical studens' awareness and knowledge levels regarding syphilis disease

Tıp Fakültesi öğrencilerinin sifilis hastalığı ile ilgili farkındalıklarının ve bilgi düzeylerinin değerlendirilmesi

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Syphilis knowledge among medical students
Received: 12.03.2025
Accepted: 31.05.2025
Doi: 10.18614/dehm.1656288

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BACKGROUND
The incidence of syphilis, a disease with severe long-term morbidities if untreated, has been increasing. This trend mainly concerns young generations, such as medical students, who are at risk. This study aimed to evaluate the knowledge and awareness levels of medical students about syphilis.
METHODS
This cross-sectional descriptive study was conducted between 01 May and 31 July 2024 at Dokuz Eylul University Faculty of Medicine. The study population consisted of medical faculty students who agreed to participate. After obtaining written informed consent, an online questionnaire was administered to students from all years of the medical faculty. The questionnaire, which was created through a literature review, included demographic questions as well as questions about sexual behavior, STD history, and prevention methods. Students were asked to rate their knowledge of the transmission routes, clinical symptoms, diagnostic procedures, treatment, and prevention methods of syphilis on a scale from 1 to 5 and to answer seven multiple-choice questions to assess their knowledge level.
RESULTS
A total of 265 students participated, with a median age of 22 years (21-23), and 52.5% (n = 134) were male. Of the students, 48.3% were sexually active and 28.3% did not use any protection method, while 51.7% used condoms. Median self-rated knowledge scores were: transmission 3 (2.5-4), symptoms 3 (2-4), diagnosis 2 (1-3), treatment 2 (1-3), and prevention 3 (2-4). The total median score was 14 (10-17). Among knowledge questions, 80.4% identified the causative agent, while correct response rates for transmission, symptoms, diagnosis, treatment, prevention, and HIV screening were 57.7%, 15.5%, 14.3%, 54.7%, 55.1%, and 57%, respectively. The median number of correct answers across all questions was 4 (2-5), with a total correct answer rate of 47.8%.
CONCLUSION
Medical students exhibit critical gaps in syphilis knowledge, with correct response rates below 50%. Given the high rate of sexual activity and inconsistent condom use, enhanced educational interventions are urgently needed to ensure both personal protection and future professional competence.
KEYWORDS
Medical students, syphilis, sexually transmitted diseases

ÖZ
AMAÇ
Tedavi edilmediğinde ciddi uzun dönem morbiditelere yol açabilen sifilis hastalığının insidansı artmaktadır. Bu eğilim, risk altında olan genç kuşaklar, özellikle tıp fakültesi öğrencileri açısından endişe vericidir. Bu çalışmada, tıp fakültesi öğrencilerinin sifilis hakkındaki bilgi ve farkındalık düzeylerinin değerlendirilmesi amaçlandı.
GEREÇ YÖNTEM
Bu kesitsel tanımlayıcı çalışma, 01 Mayıs–31 Temmuz 2024 tarihleri arasında Dokuz Eylül Üniversitesi Tıp Fakültesi'nde gerçekleştirildi. Çalışma popülasyonunu katılmayı kabul eden tıp fakültesi öğrencileri oluşturdu. Yazılı onam alındıktan sonra, tıp fakültesinin tüm sınıflarındaki öğrencilere çevrimiçi bir anket uygulandı. Literatür taraması ile oluşturulan anket, demografik bilgilerin yanı sıra cinsel davranışlar, CYBH öyküsü ve korunma yöntemleri ile ilgili sorular içeriyordu. Öğrencilerden, sifilisin bulaş yolları, klinik belirtileri, tanı yöntemleri, tedavisi ve korunma yöntemlerine ilişkin bilgi düzeylerini 1'den 5'e kadar puanlamaları ve bilgi düzeylerini ölçmek için yedi çoktan seçmeli soruyu yanıtlamaları istendi.
BULGULAR
Çalışmaya toplam 265 öğrenci katıldı; ortanca yaş 22 (21-23) olup %52,5'i (n = 134) erkekti. Katılımcıların %48,3'ü cinsel olarak aktif olduğunu, %28,3'ü herhangi bir korunma yöntemi kullanmadığını ve %51,7'si prezervatif kullandığını belirtti. Öğrencilerin kendi puanladıkları bilgi düzeylerinin ortanca değerleri şöyleydi: Bulaş yolları 3 (2,5-4), klinik belirtiler 3 (2-4), tanı yöntemleri 2 (1-3), tedavi 2 (1-3) ve korunma yöntemleri 3 (2-4). Toplam ortanca puan 14 (10-17) idi. Bilgi sorularında; katılımcıların %80,4'ü hastalığın etkenini doğru yanıtladı. Bulaş yolları, klinik belirtiler, tanı, tedavi, korunma ve HIV taraması ile ilgili doğru yanıt oranları sırasıyla %57,7, %15,5, %14,3, %54,7, %55,1 ve %57 olarak bulundu. Tüm sorular için ortanca doğru yanıt sayısı 4 (2-5) olup toplam doğru cevap oranı %47,8 idi.
SONUÇ
Tıp fakültesi öğrencilerinin sifilis bilgileri kritik düzeyde yetersizdir ve doğru yanıt oranları %50'nin altındadır. Cinsel aktiflik oranının yüksek ve prezervatif kullanımının düzensiz olduğu göz önüne alındığında hem kişisel korunma hem de gelecekteki mesleki yeterlilik açısından eğitim müdahalelerinin artırılması acil bir ihtiyaçtır.
ANAHTAR KELİMELER
Cinsel yolla bulaşan hastalıklar, frengi, tıp öğrencileri

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Syphilis is a systemic infection caused by the spirochete *Treponema pallidum subspecies pallidum*. It can be transmitted through blood, sexual contact, direct contact with active skin lesions, or transplacentally (1). Despite the availability of effective treatment, syphilis remains a significant public health issue, and its incidence has been increasing globally. In the United States (USA), in 2023, the highest number of cases since the 1950s was reported, with 209,253 cases showing an increase in frequency among women, men, and all age groups (2). According to the Turkish Ministry of Health data, the incidence of syphilis in Türkiye has been rising, with a sevenfold increase in confirmed syphilis cases in 2023 compared to 2015. In 2023, the incidence rate was recorded at 4.27 cases per 100,000 population, with the majority of cases being in men (3). This increase is thought to be influenced by changes in sexual behavior patterns, the spread of premarital sexual activity, and the increased availability of applications for accessing sexual partners via smartphones, leading to an increase in the number and diversity of partners (4).

Co-infection with the human immunodeficiency virus (HIV) and syphilis is common due to their similar transmission routes, where one infection can facilitate the transmission of the other (5). In previous years, easier access to HIV control programs and prevention materials provided significant protection against other sexually transmitted infections. However, recent advancements in HIV treatment and the widespread use of pre-exposure prophylaxis (PrEP) have resulted in reduced use of barrier methods, such as condoms, creating a false sense of security. This decline in condom use has been identified as a contributing factor to the increased frequency of syphilis, especially among at-risk groups (6-7).

While the exact prevalence of syphilis among university students in Türkiye is not well known, official data indicates that in 2023, approximately 16% of syphilis cases occurred among adolescents and young adults aged 15-25 years (3). In a multi-center study conducted in Türkiye, which investigated HIV-syphilis co-infections, 8% of the cases were among young adults aged 18-24, and 3% were students (8). Although sexual activity is common among university students, studies have shown that condom use, the most effective method of preventing syphilis, is low, and knowledge and awareness about syphilis remain insufficient (9-10). Although several small studies have been conducted in recent years regarding students' awareness of sexually transmitted diseases (STDs) in Türkiye, no studies focusing on syphilis have been found (11-13). This study aimed to assess the knowledge and awareness of medical students about syphilis, a disease that can result in severe long-term complications if left untreated.

Materials and Methods

Our study was conducted as part of the Special Study Module for the 3rd-year students of the Dokuz Eylul University Faculty of Medicine (DEUFM) and is a descriptive-cross-sectional type of research. The study population consists of students from DEUFM, including those from the 1st, 2nd, 3rd, 4th, 5th, and 6th grades who were over 18 years old and had agreed to participate. There is no standardized questionnaire that has been validated and tested for reliability to measure awareness and/or knowledge levels about syphilis among university students. Therefore, after reviewing the literature, a questionnaire form was created (1, 14-17). The questionnaire was applied using an online survey technique after obtaining online written consent from the medical students between 01 May and 31 July 2024. The link to the questionnaire was shared through the students' email addresses and social media groups, and they were asked to complete it within two months. Weekly reminder messages and the questionnaire link were sent during the two months. Additionally, in face-to-face meetings, the online questionnaire was filled out by scanning a QR code.

Demographic information, such as age, gender, year of study in medical school, parental education, and income status, was collected from the participants, along with questions regarding sexual behavior, STD history, and prevention methods. Students were asked to evaluate their knowledge levels regarding syphilis transmission routes, clinical symptoms, diagnostic methods, treatment, and prevention methods by scoring themselves on a scale of 1 to 5 (1: I do not know at all, 2: I think my knowledge is insufficient, 3: I am not sure, 4: I know, 5: I definitely know). Additionally, students were asked to answer seven multiple-choice knowledge questions.

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Statistical analysis

Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS), version 26.0 (IBM Corp., Armonk, NY, USA) software. The normality of continuous variables was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Categorical variables were expressed as counts and percentages, while continuous variables were expressed as medians (interquartile range). The students were grouped into lower years (1st, 2nd, and 3rd years) and upper years (4th, 5th, and 6th years), and the scores and the number of correct answers between the lower and upper years were compared using the Mann-Whitney U test and chi-square test. A p-value of less than 0.05 was considered statistically significant.

Limitations of the research

The study population consisted exclusively of medical students from a single institution. The survey was administered primarily through electronic means, and voluntary participation introduced the potential for selection and social desirability biases. The questionnaire was mainly distributed electronically (via email, social media, and QR code), which may have excluded students with limited access or reduced motivation. Participants' values and sensitivities may have influenced the responses regarding sexual behaviors and preventive practices. The questionnaire used to assess knowledge and awareness was also developed based on a literature review, but was not subjected to formal validation or reliability testing. Part of the data relied on self-assessment using a subjective 1–5 Likert scale. Self-perceived knowledge may not accurately reflect actual knowledge levels.

Ethical approval

The research was initiated after obtaining ethical committee approval for non-interventional studies (Decision No: 2024/16-42, Date: 08/05/2024).

Results

The questionnaire was distributed via email to approximately 1,500 students and shared on the social media platforms of six different class groups. Although the exact number of duplicate entries could not be determined, 265 students completed the form and participated in the study. Accordingly, the estimated response rate was approximately 17.7%.

The median age of the students was 22 years (range 21-23 years), with 52.5% (n = 134) of the participants being male. Participants were distributed as follows: 1st year (3.8%, n = 10), 2nd year (11.3%, n = 30), 3rd year (25.7%, n = 68), 4th year (30.6%, n = 81), 5th year (22.6%, n = 60), and 6th year (6%, n = 16). Regarding the education level of the students' parents, 48.3% (n = 128) had a bachelor's degree, 17.4% (n = 46) had a master's degree, 17% (n = 45) had a high school diploma, 9.8% (n = 26) had an elementary school diploma, and 7.5% (n = 20) had a middle school diploma. When evaluating the income levels of the parents, 38.9% (n = 103) had an income between TRY 17,000-50,000 , 37% (n = 98) had an income between TRY 50,000-100,000, 14.3% (n = 38) had an income of TRY 100,000 or more, and 9.8% (n = 26) had an income below TRY 17,000.

Regarding sexual activity, 48.3% (n = 128) of the participants reported being sexually active, while 39.6% (n = 105) stated they had never had sexual intercourse. Thirty-two (12%) participants preferred not to share information about their sexual activity. Among the students, 20.4% (n = 54) reported that they had been tested for sexually transmitted infections (STIs), and 1.5% (n = 4) indicated that they had contracted an STI. In terms of protection from STIs, 28.3% (n = 75) of the students did not use any protection, while 51.7% (n = 137) used condoms. Among the sexually active students, 9.4% (n = 12) did not use any form of protection, while 82.7% reported using condoms.

In terms of knowledge levels, students rated their understanding of various aspects related to syphilis as follows: knowledge about transmission routes received a median score of 3 (ranging from 2.5 to 4), clinical symptoms also scored a median of 3 (with a range of 2 to 4), diagnostic tests received a median score of 2 (ranging from 1 to 3), treatment scored a median of 2 (with a range of 1 to 3), and prevention methods achieved a median score of 3 (ranging from 2 to 4). The overall median score was 14 (range: 10–17) (Table 1).

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Table 1. Medical students' self-assessment of their knowledge levels regarding syphilis transmission routes, clinical findings, diagnostic tests, treatment, and prevention methods

	1: I do not know at all (%)	2: I think my knowledge is insufficient (%)	3: I am unsure (%)	4: I know (%)	5: I definitely know (%)	Median range, (IQR)
Transmission route[a]	23 (8.7)	43 (16.2)	92 (34.7)	87 (32.8)	20 (7.5)	3 (2.5-4)
Clinical symptoms[b]	42 (15.8)	54 (20.4)	94 (35.5)	52 (19.6)	23 (8.7)	3 (2-4)
Diagnostic tests[c]	69 (26)	69 (26)	77 (29.1)	32 (12.1)	18 (6.8)	2 (1-3)
Treatment[d]	72 (27.2)	65 (24.5)	71 (26.8)	28 (10.6)	29 (10.9)	2 (1-3)
Prevention[e]	36 (13.6)	64 (24.2)	79 (29.8)	52 (19.6)	34 (12.8)	3 (2-4)

[a] Rate your knowledge of syphilis transmission routes on a scale of 1 to 5.
[b] Rate your knowledge regarding the clinical findings of syphilis on a scale of 1 to 5.
[c] Rate your knowledge regarding the diagnostic tests for syphilis on a scale of 1 to 5.
[d] Rate your knowledge regarding the treatment of syphilis on a scale of 1 to 5.
[e] Rate your knowledge regarding the prevention methods for syphilis on a scale of 1 to 5.
IQR=Interguantile Range

When responding to knowledge questions, the students demonstrated the following levels of understanding: 80.4% correctly identified the causative agent of syphilis, while 57.7% accurately answered questions regarding transmission routes. Additionally, 15.5% correctly responded to questions about clinical symptoms, and 14.3% accurately answered diagnostic test questions. Furthermore, 54.7% provided correct answers related to treatment, 55.1% responded correctly to questions about prevention, and 57% accurately answered questions concerning HIV screening. The median number of correct answers for all questions was 4 (range 2-5), and the overall correct answer rate was 47.8%.

When comparing the correct answers provided by lower-year students (1st, 2nd, and 3rd years) and upper-year students (4th, 5th, and 6th years), both groups showed the highest correct answer rate for the question about the causative agent of syphilis, with no statistically significant difference between the two groups ($p = 0.569$). Conversely, the lowest correct answer rates in both groups were for questions regarding the clinical symptoms of syphilis and the microbiological methods used for diagnosis. While there was no significant difference in correct answers for clinical symptoms ($p = 0.104$), the rate of correct answers regarding laboratory diagnosis was significantly higher among lower-year students than upper-year students ($p = 0.020$). Overall, the median number of correct answers for knowledge questions was 3 (range: 2-4) for lower-year students and 4 (range: 3-5) for upper-year students, with a significant difference between the two groups ($p < 0.001$) (Table 2).

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Table 2. Correct response rates to knowledge questions on syphilis transmission routes, clinical findings, diagnostic tests, treatment, and prevention methods among medical students

	The correct answers of lower-year students (Years 1- 2, and 3), n = 108 (40.8%)	**The correct answers of upper-year students (Years 4- 5, and 6), n = 157 (59.2%)**	**p-value**
Demographic and sexual data			
Age, median age (IQR)	21 (20-22)	23 (22-23)	<0.001
Gender			
Male	59 (54.6)	80 (51)	0.556
Female	49 (45.4)	77 (49)	
Sexual activity	54 (50)	74 (47.1)	0.646
Condom use	55 (56.7)	82 (55.8)	0.835
Non-use of Contraception	35 (36.1)	40 (27.2)	0.219
HPV vaccination	3 (3.1)	7 (4.8)	0.721
Self-rating, median score (IQR)			
Transmission route[a]	3 (2-4)	3 (3-4)	0.093
Clinical symptoms[b]	3 (2-4)	3 (2-4)	0.103
Diagnostic tests[c]	2 (1-3)	2 (2-3)	0.499
Treatment[d]	2 (1-3)	3 (2-3)	0.018
Prevention[e]	3 (2-4)	3 (2-4)	0.800
Correct answer counts and percentages for knowledge questions			
Which of the following are modes of transmission of syphilis? (Check all the correct options) a) Sexual transmission (Oral, anal, or vaginal intercourse) b) Transmission via the respiratory route c) Transmission through sharing personal items d) Transmission via blood e) Maternal transmission (From mother to baby during pregnancy) Correct answer: a, d, e	48 (44.4)	105 (66.9)	< 0.001

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Which of the following is not a clinical symptom of syphilis? a) Genital ulcer - chancre b) Inguinal lymphadenopathy c) Uveitis d) Skin rash - maculopapular rash e) Genital discharge Correct answer: e	12 (11.1)	29 (18.5)	0.104
Which of the following is the causative agent of syphilis? a) *Treponema pallidum* b) *Borrelia burgdorferi* c) *Haemophilus ducrei* d) *Neisseria gonorea* e) *Chlamydia pneuomophila* Correct answer: a	85 (78.7)	128 (81.5)	0.569
Which of the following diagnostic methods are used for syphilis? a) RPR b) VDRL c) TPPA d) FTA-ABs e) EIA-Syphilis antibody f) *T. pallidum* PCR g) Darkfield microscopy Correct answer: All of them are correct	22 (20.4)	16 (10.3)	0.020
Which of the following is the first-line treatment for primary syphilis? a) There is no treatment b) A single dose of 2.4 million units of benzathine penicillin c) Doxycycline 2x100 mg for 1 week d) Ceftriaxone 2x2 g IV for 14 days e) Moxifloxacin 1x400 mg for 1 week Correct answer: b	39 (36.1)	106 (67.5)	<0.001

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Which of the following statements about the prevention of syphilis is incorrect? a) Regular and proper condom use prevents the transmission of syphilis, as well as other sexually transmitted infections. b) Receiving two doses of a vaccine against *T. pallidum* prevents syphilis. c) Individuals at high risk for syphilis should undergo screening at least once a year. d) Pregnant women should be screened for syphilis during their first prenatal visit, which is effective in preventing congenital syphilis. e) Individuals diagnosed with syphilis should inform their sexual partners and refer them for testing to prevent new infections. Correct answer: b	52 (48.1)	94 (59.9)	0.059
Patients diagnosed with syphilis should be tested for which of the following sexually transmitted infections?" a) Human immunodeficiency virus (HIV) b) Human papilloma virus (HPV) c) Human herpes virus 2 (HSV-2) d) *Haemophilus ducreyi* e) *Trichomonas vaginalis* Correct answer: a	56 (51.9)	95 (59.2)	0.162
Total score, median (interquartile range)	3 (2-4)	4 (3-5)	< 0.001

[a]Rate your knowledge of syphilis transmission routes on a scale of 1 to 5.
[b] Rate your knowledge regarding the clinical findings of syphilis on a scale of 1 to 5.
[c] Rate your knowledge regarding the diagnostic tests for syphilis on a scale of 1 to 5.
[d] Rate your knowledge regarding the treatment of syphilis on a scale of 1 to 5.
[e] Rate your knowledge regarding the prevention methods for syphilis on a scale of 1 to 5.
IQR = Interquartile Range; **HPV** = Human papillomavirus

Discussion

In our study, medical students' knowledge and awareness levels regarding syphilis were evaluated, and the rate of correct answers to the knowledge questions was below 50%. In their self-assessments, the students gave themselves a median score of 3, meaning "I am not sure," indicating low awareness. In a study conducted in the United States, an online survey consisting of 25 questions was administered to 231 participants, of whom 45% were medical students. The correct answer rate was found to be 33% (18). The insufficient knowledge of medical students regarding syphilis is thought to be due to the lack of theoretical and/or practical education on syphilis in the medical curriculum. In Bonnewell et al.'s study, only 27% of students reported receiving sufficient

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education on syphilis (18). A different study from Brazil found that 64.7% of students from various faculties had an adequate level of knowledge about syphilis (9). Yıldırım and Erbil, in a systematic review of 12 studies assessing the knowledge levels of university students in Türkiye regarding STDs, reported that students enrolled in health-related departments generally had a moderate to adequate level of knowledge (19). The differences between studies are likely due to differences in study design, the content of the surveys, and educational, social, and cultural differences between countries.

The self-assessed knowledge scores of students were relatively higher in transmission routes and prevention methods. In contrast, their knowledge about clinical processes such as diagnosis and treatment was observed to be lower and was considered inadequate. Similarly, in the literature, it has been reported that students' knowledge of diagnosis and treatment is lower compared to other areas, and this is attributed to the fact that medical education tends to emphasize theory over practice (18). The lack of knowledge regarding microbiological methods used in syphilis diagnosis and treatment options suggests that the educational curriculum in this area should be strengthened. Potential improvements to the medical curriculum could include more practical training in diagnosis and treatment, as well as a greater emphasis on the microbiological aspects of syphilis, to ensure that students are adequately prepared to manage this and other sexually transmitted infections in their future clinical practice.

When comparing the knowledge levels of lower and upper-year students, it was observed that upper-year students had higher knowledge levels. Still, this difference did not fully compensate for the general lack of knowledge. Specifically, the higher correct answer rate of lower-year students regarding diagnostic methods for syphilis might be attributable to the effectiveness of early basic microbiology education. Still, the integration of this knowledge into clinical practice is insufficient. A study in the United States evaluated medical students' knowledge of sexual health and found that upper-year students had significantly higher knowledge levels. Additionally, students who took a human sexuality course in the medical curriculum showed higher knowledge levels (20). A recent study conducted in Türkiye demonstrated that training on STDs effectively increased students' knowledge levels. According to the research by Tetik Metin et al. (2025), first-year students initially had significantly lower scores on STD knowledge compared to second-year students ($p < 0.05$). However, after the training, no significant differences were found between the two class levels ($p > 0.05$) (21).

In our study, the prevalence of sexual activity among students was approximately 50%, and 80% of this group reported using condoms, indicating awareness of prevention methods for sexually transmitted infections (STIs). However, 28.3% of students did not use any protection, highlighting the need for more comprehensive education. Studies from various countries have reported sexual activity rates among university students ranging from 60% to 80% (22,23). During this transitional period from adolescence to adulthood, university students, especially those living away from their families, have greater freedom, which may increase the frequency of sexual experiences. Moreover, a lack of knowledge and expertise regarding STIs, multiple sexual partners, and the adoption of risky habits such as alcohol and drug use may lead to risky sexual behaviors (9). A study conducted in Serbia reported that students in both medical and non-medical faculties had limited knowledge about the undesirable consequences of risky sexual behaviors (24). In Mexico, another study found that although condom use during vaginal sex among medical students was high (75%), condom use during oral and anal sex was lower (20). It is essential to increase medical students' knowledge to protect themselves from STIs like HIV and syphilis, to serve as role models for their peers, to contribute to peer education, and to provide adequate healthcare services related to STIs during their future clinical practices.

This study is the first to evaluate syphilis awareness and knowledge levels among medical students in Türkiye, and no other national-level research on this topic was found. Therefore, it is considered an original study and a valuable contribution to the literature. However, the study has some limitations. The study population is limited to medical students from our university, so the results may not fully represent medical students across Türkiye. Additionally, the survey was primarily conducted electronically, and participation was voluntary. This may have introduced the risk of social desirability bias. The accuracy of answers, particularly regarding sexual behaviors and preventive methods, may have been influenced by the participants' values and sensitivities.

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Finally, the validity and reliability of the knowledge questions used in the study were not tested. Although the questions were prepared based on the literature, the lack of standardization may limit the comparability of the results. Future studies could benefit from developing or using a standardized survey with international validity to overcome such limitations. This highlights the potential for further research in this area and the need for more comprehensive studies to fully understand the knowledge and awareness levels of medical students regarding syphilis.

Conclusion

This study highlights that the lack of syphilis knowledge among medical students is a significant issue both for their personal protection and their future clinical practices. Approximately 50% of medical students were sexually active, and 80% of them reported using condoms as a preventive measure. However, students assessed their knowledge of syphilis as insufficient, with a correct answer rate below 50%. This has significant implications for their future clinical practices. To protect themselves, guide their peers and the community, and manage accurate diagnosis and treatment processes in their future clinical practice, medical students must improve their knowledge levels and gain sufficient confidence in this area. The findings of this study underscore the importance of early and comprehensive educational programs on sexually transmitted infections, particularly syphilis, in medical education. Furthermore, awareness campaigns targeting students and enriching the academic curriculum will help students become more equipped in personal protection and professional competence, thereby improving public health outcomes.

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Single center surgical complications in vagus nerve stimulation surgery

Vagal sinir stimülasyon cerrahisinde tek merkezli cerrahi komplikasyonlar

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Surgical Complications in Vagus Nerve Stimulation Surgery
Received: 28.03.2025
Accepted: 01.06.2025
Doi: 10.18614/dehm.1667545

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BACKGROUND
Vagal nerve stimulation surgery is one of the prominent neuromodulation methods due to its increasing spectrum of indications and number of cases worldwide. Surgical complications of this procedure may lead to temporary or permanent results and may require repeated surgeries. This study aimed to compile the surgical complications of patients who underwent vagus nerve stimulation surgery.
METHODS
Adult patients between the ages of 18 and 65 who underwent vagus nerve stimulation surgery in a single center were grouped as initial implantation, pulse generator revision, complete revision, or system removal. Average surgery time was documented, along with age and gender information. Data on surgery-related complications such as local infection, headache, cough, temporary or permanent hoarseness, vascular injury and hematoma were examined in all patients.
RESULTS
There were 44 patients subjected to the study. The most frequently performed procedure was primary implantation in 77.3% of the patients (n : 34). One or more complications developed in 29.54% (n : 13). The most common surgical complication was headache in 7 patients (15.9%), and the only permenant complication was hoarseness in 1 patient (2.9%).
DISCUSSION
Vagus nerve stimulation surgery improves the patient's quality of life. Life threatening and permanent complications are not common in vagus nerve stimulation surgery. Our surgical complication rates are similar to those reported in the literature.
CONCLUSION
Vagus nerve stimulation surgery is a relatively safe procedure. Further research will help to better understand complications and rates.
KEYWORDS
Epilepsy, surgical complications, vagus nerve stimulation

ÖZ

AMAÇ
Vagal sinir stimülasyonu cerrahisi, dünya genelinde artan endikasyon spektrumu ve vaka sayıları nedeniyle öne çıkan nöromodülasyon yöntemlerinden biridir. Bu işlemin cerrahi komplikasyonları geçici veya kalıcı sonuçlar doğurabilmekte, tekrarlayan cerrahiler gerektirebilmektedir. Bu çalışmada vagus sinir stimülasyonu cerrahisi uyguladığımız hastaların cerrahi komplikasyonlarının derlenmesi amaçlanmıştır.
GEREÇ YÖNTEM
Tek merkezde vagus sinir stimülasyonu cerrahisi yapılan 18-65 yaş aralığındaki erişkin hastalar ilk implantasyon, pil değişimi, VNS sistem revizyonu ve VNS sisteminin tamamen çıkarılması olarak gruplandırıldı. Yaş ve cinsiyet bilgileri ile birlikte ortalama cerrahi süresi dokümente edildi. Tüm hastalarda cerrahi ilintili komplikasyonlar olan lokal enfeksiyon, baş ağrısı, öksürük, geçici veya kalıcı ses kısıklığı, vasküler yaralanma ve hematom komplikasyonları verileri incelendi.
BULGULAR
Çalışmaya 44 hasta dahil edildi. En sık yapılan işlemin %77,3 hastada (n : 34) primer implantasyon olduğu görüldü. Hastaların %29,54'ünde (n : 13) bir veya daha çok komplikasyon geliştiği, en sık cerrahi komplikasyon 7 hastada (%15,9) baş ağrısı, düzelmeyen tek komplikasyonun 1 hastada (%2,9) kalıcı ses kısıklığı olduğu izlendi.
SONUÇ
Vagus sinir stimülasyonu hastaların yaşam kalitesini artırmaktadır. Yaşamı tehdit eden veya kalıcı komplikasyonlar oldukça nadirdir. Vagus sinir stimülasyonundaki cerrahi komplikasyonlarımız literatürle benzerlik göstermektedir.

ANAHTAR KELİMELER
Cerrahi komplikasyonlar, epilepsi, vagus sinir stimülasyonu.

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Vagus nerve stimulation (VNS) is one of three neuromodulation methods that can be applied to patients who do not respond to pharmacological treatment and are not suitable for surgical resection (1). Since its application on humans in 1988 (2), as a result of the studies carried out over the years, it has also begun to be used in the palliative treatment of major depression and headache (3,4). VNS surgery was the first to be used among neuromodulation methods. It continued to be the most frequently used method as the indication expanded and the number of cases increased (5).

VNS complications can be evaluated under two headings: surgical and hardware-related complications. Surgical complications are infection, headache, cough, temporary or permanent hoarseness, vascular injury, and cervical hematoma. Hardware related complications are lead fracture (especially in children), disconnection, spontaneous turn-off, and stimulator malfunction (6).

This study aimed to examine the surgery-related complications seen in patients who underwent VNS surgery due to drug-resistant epilepsy.

Materials and Methods

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Bakirçay University Clinical Research Ethics Committee (dated 07/03/2025 and decision number 2093). Adult patients between the ages of 18-65, who were operated on by the same surgical team in the same hospital, between February 2022 and February 2025 were retrospectively examined. Age and gender information was documented through archive scanning. Patients who underwent surgery were grouped as primary implantation, pulse generator revision, complete system revision or complete removal of the system, and data on surgery-related complications such as local infection, headache, cough, temporary or permanent hoarseness, vascular injury, and hematoma were collected in all patients.

Statistical analysis

All records and data regarding the patients were analyzed with the SPSS 23.00 statistical package program. Data were examined with descriptive statistics (number, percentage distribution, mean, standard deviation).

The average age of the 44 patients included in the study was 31.97± 5.64. 56.8% (n : 25) of the patients were female and 43.2% (n : 19) were male. 77.3% (n : 34) underwent primary implantation, 18.2% (n : 8) underwent pulse generator revision, 1 patient (2.3%) underwent complete revision, and 1 (2.3%) patient underwent system removal.

The average surgical time for the procedures was calculated as 79.54± 24.67 minutes (Table 1).

	Primary implantation (n : 34)	Pulse generator revision (n : 8)	Complete revision (n : 1)	System removal (n : 1)
Gender, n (%)	21 (61.8%)	3 (37.5%)	1 (100%)	-
Female	13 (38.9%)	5 (62.5%)	-	1 (100%)
Male				
Age	31.29 ± 5.61	33.87 ± 5.79	-	-
Surgery duration (minutes)	92.05 ± 8.8	37.5 ± 4.62	140	55

Table 1. Patient characteristics by procedure

29.54% (n : 13) of the patients developed one or more complications, and 6.8% (n : 3) of these complications were local infection, 15.9% (n : 7) were headache, 11.4% (n : 5) were cough, 11.4% (n : 5) were temporary hoarseness, and 2.9% (n : 1) were permanent hoarseness. No vascular injury or hematoma was observed in any patient (Table 2).

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Table 2: Complications by procedure

	Primary implantation (n : 34)	Pulse generator revision (n : 8)	Complete revision (n : 1)	System removal (n : 1)
Local infection, n (%)	3 (8.8%)	-	-	-
Headache, n (%)	7 (15.9%)	-	-	-
Cough, n (%)	5 (11.4%)	-	-	-
Temporary hoarseness, n (%)	5 (11.4%)	-	-	-
Permanent hoarseness, n (%)	1 (2.9%)	-	-	-

The characteristic features of the patients grouped according to the surgical procedure and the complications that developed are given in Table 1 and 2. Due to sample sizes of 5 or less in group distributions, only descriptive statistics were evaluated. All of the complications that occurred in the patients occurred during primary implantation surgery. No complications were observed after other surgical procedures.

Discussion

This single-center study evaluated a group of 44 surgeries that were performed from February 2022 to February 2025. 34 were primary implantation, 8 were pulse generator revision, 1 was complete revision and 1 was partial removal of the pulse generator and the lead leaving approximately 3 cm behind including the electrode coils surrounding the nerve. Surgical complication rate for primary VNS implantation was 20.5%, including 7 patients with one or multiple complications out of 34.There were no complications in pulse generator revision, system complete revision or system removal. This study found a total complication rate of 15.9%, which correlates previous literature that showed surgical complication rates ranging from 4.2% to 16.7% (7,8). There were also no cardiac side effect,no hematoma, and no vascular injury in this cohort.

VNS surgeries improve the patient's quality of life (9). Life threatening complications are not common in VNS surgery. Permanent failure of the recurrent laryngeal nerve, which led to left-sided vocal cord paresis and hoarseness, only occurred in one patient, who was a secretary talking with telephones in a school. She also had headache and local infection in pulse generator area. Local infection was controlled with antibiotics and local debridement of the surgical incision in approximately 4 weeks. In this particular patient, VNS had a good seizure-suppressing effect, making the occurrence of this complication acceptable for the patient. On the other hand, a male patient in his 30s could not adapt to the pulse generator due to pre-existing behavioral problems. Signs of local infection and non-closure were observed in both wounds because he constantly scratched both incisions. Both wounds were debrided, complete revision of the system was performed, and antibiotic therapy was continued in the first week. The patient's compliance problem did not change. Since the wounds did not close and the VNS system could not be turned-on, its effect on epilepsy control could not be determined. The generator and electrode coil system were removed upon the request of the family. No signs of deep infection were observed during peroperative observation. The helical coils adhered to the vagus nerve with fibrosis within 1 month, so this part was cut and left. There were no postoperative surgical complications. The patient had no compliance problems and the incisions were closed after the system was removed.

Mean surgery duration was found to be statistically significant risk factor by J. van Schooten et al. Mean surgery duration was longer for patients with complications after lead revision (150.71 min) compared to patients without complication (123.64 min) (7). There is 1 patient outgoing to lead revision which was 140 minutes of surgery time. There was no surgical complication about this patient. Due to the low number of lead revision sample size, it would not be appropriate to make a comparison for this risk factor.

Conclusion

This single-center retrospective study shows that VNS implantation is a relatively safe procedure. Our surgical complication rates are similar with literature. Further research will help to better understand complications and rates.

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Limitations

The retrospective design is the major limitation. The low number of cases constitutes another limitation for statistics, as these surgical interventions are not yet widely performed in every center and are used only in drug resistant epilepsy patients. Due to the small sample size inof the pulse generator revision, complete revision, and system removal groups, no formal statistical analysis could be performed for these.

Patient consent

Patient consent was obtained from all subjects involved in this study.

Conflict of interest

The athors declare that there is no conflict of interest.

Acknowledgements

The authors declare that there is no acknowledgement.

Author contribution

Concept and Design: GG; Analysis and Interpretation: GG, GG; Data Collection: GG; Manuscript Writing: GG; Review and Editing: GG, GG; Approval: GG, GG

Data availability

Data available upon request from corresponding author due to legal/ethical reasons.

Funding

This research didn't receive grants from any funding agency in the public, commercial or not-for-profit sectors.

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Review of carcinogenesis mechanisms and new therapeutic targets in colorectal cancer

Kolorektal kanserde karsinogenez mekanizmalarının ve yeni tedavi hedeflerinin gözden geçirilmesi

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Review of Carcinogenesis Mechanisms and New Therapeutic Targets in Colorectal Cancer
Received: 05.11.2024
Accepted: 30.01.2025
Doi: 10.18614/dehm.1751154

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ABSTRACT

Colorectal cancer (CRC) is the third most common cancer among women and men worldwide. The risk of developing CRC is influenced by both environmental and genetic factors. It is known that more than one carcinogenesis mechanism occurs sequentially in some cancers, especially colorectal cancer. In this review, it is planned to present the altered signaling pathways specific to colorectal carcinogenesis and related mechanisms of carcinogenesis and to reveal possible new treatment targets.

KEYWORDS

Colorectal cancer, multistep carcinogenesis

ÖZ

Kolorektal Kanser (KRK)'ler tüm dünyada kadınlar ve erkeklerde görülen kanserler arasında üçüncü en sık gözlenen kanserdir. KRK gelişme riski hem çevresel hem genetik faktörlerden etkilenir. Kolorektal kanser başta olmak üzere bazı kanserlerde birden çok karsinogenez mekanizmasının ardışık olarak ortaya çıktığı bilinmektedir. Bu derlemede kolorektal karsinogenez özelinde değişen sinyal yolakları ve ilişkili karsinogenez mekanizmalarının güncel olarak gözden geçirilerek sunulması ve olası yeni tedavi hedeflerinin ortaya konması planlanmıştır.

ANAHTAR KELİMELER

Çok aşamalı karsinogenez, kolorektral kanser, sinyal yolakları

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The transformation of a normal cell into a cancer cell requires the acquisition of multiple genetic alterations within the cell. This process, known as carcinogenesis, is a multistep process involving the influence of multiple genes (1). The activation of various proto-oncogenes and the inactivation of tumor suppressor genes lead to progressive changes in the morphological appearance of normal epithelial tissue. These changes result in dysplasia, adenoma, or intraepithelial premalignant lesions, eventually culminating in invasive carcinoma. Throughout this developmental process, numerous oncogenic pathways frequently undergo alterations, which have been described by Hanahan and Weinberg as the "hallmarks of cancer." These hallmarks include: independence from growth-regulating signals (e.g., constitutive activation of K-RAS through point mutations; mutations in p53 or Rb), defects in apoptosis (e.g., overexpression of Bcl-2), tissue remodeling and metastasis (e.g., ß-catenin/WNT), unlimited proliferation (e.g., activation of telomerase), and the induction of neoangiogenesis (e.g., autocrine or paracrine secretion of vascular endothelial growth factor (VEGF). Additional hallmarks include the reprogramming of energy metabolism (the Warburg effect), evasion of the immune system, inflammation that promotes cancer development, genomic instability and accumulating mutations, phenotypic plasticity and impaired differentiation, non-mutational epigenetic reprogramming, the influence of microbiome, and the presence of senescent cells (2). Most of these changes are also observed in colorectal cancer.

Colorectal cancer (CRC)

Colorectal cancer (CRC) is the third most common cancer worldwide, accounting for approximately 10% of all cancer cases. It is the second leading cause of cancer-related deaths globally. In 2020, over 1.9 million new CRC cases and more than 930.000 deaths from colorectal cancer were estimated to occur worldwide. By 2040, the CRC burden is expected to rise to 3.2 million new cases annually (a 63% increase) and 1.6 million deaths annually (a 73% increase) (3). The 5-year survival rate for patients diagnosed with early-stage (stage I and stage II) and localized CRC is nearly 90% (4). In contrast, if diagnosed at more advanced or metastatic stages, the survival rate drops to only 13.1%. CRC develops in different anatomical regions based on the patient's gender. In women, CRC is more commonly found in the right colon, whereas in men, it is more often found in the left colon. Men are more likely to develop metastatic colon cancer, while women tend to develop metastatic rectal cancer as they age (5). Colorectal cancer affects the colon (large intestine) or rectum. Most CRCs begin as polyps on the inner lining of the colon or rectum. The most common type of colon cancer, adenocarcinomas (about 90%) (6), develop from adenomas or adenomatous polyps (7). Adenomas are therefore referred to as precancerous lesions. Adenomas show mild, moderate, or severe epithelial dysplasia under a microscope. Although adenomas do not have the ability to invade or spread to surrounding tissue, carcinomas display similar phenotypic characteristics (8). There is a typical progression from hyper-proliferative epithelium to dysplastic crypt foci, macroscopically distinct tubular adenoma, progressive dysplastic and/or villous adenoma, and invasive cancer. However, this transition is slow, and less than one in ten adenomas (< 1) progress to carcinoma. Adenomas are classified into three types: villous, tubular, and tubulovillous. Tubular adenomas are the most common form of adenomatous polyps. Although more common, inflammatory and hyperplastic polyps are generally not indicators of malignancy (9). Additionally, sessile serrated polyps (SSPs) and conventional serrated adenomas (TSAs) are often treated like adenomas due to their higher likelihood of transforming into cancer.

Colorectal cancers can be classified into three subtypes: familial, hereditary, and sporadic (10). Approximately 70% of CRC cases are sporadic carcinomas, which occur without a genetic or family history. In individuals over the age of 50, the likelihood of developing sporadic cancer increases to 90% due to the overlap of natural aging with environmental and dietary factors. About 85% of sporadic CRCs show chromosomal instability (CIN), which leads to changes in chromosome number and structure. These changes include the gain or loss of chromosomal segments, chromosomal rearrangements, and loss of heterozygosity (LOH), which results in gene copy number variations (CNVs) (5). These alterations affect the expression of tumor-associated genes and/or genes that regulate cell proliferation or cell cycle control points, as illustrated in Figure 1.

This, in turn, can activate the defined key pathways for CRC initiation and progression, such as the Adenomatous

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Polyposis Coli (APC), KRAS, Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), B-RAF proto-oncogene, Serine/Threonine Kinase (BRAF), SMAD4, and TP53 genes (11).

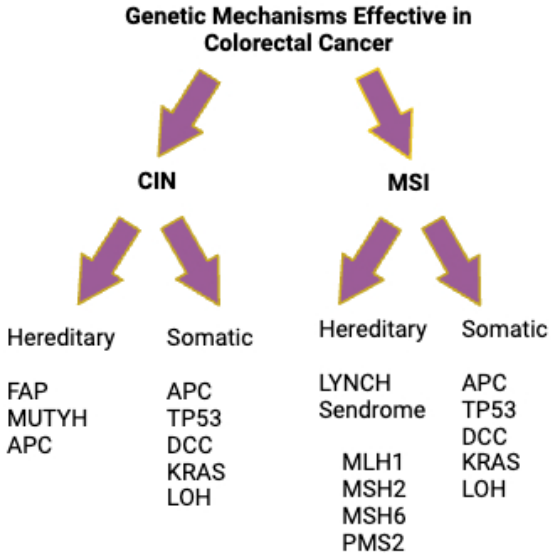

Figure 1. Genetic mechanisms involved in colorectal cancer

Approximately 15% of the remaining sporadic cases exhibit a high-frequency microsatellite instability (MSI) phenotype. Patients with a hereditary predisposition to colon cancer account for less than 10% of all cases. Hereditary colorectal carcinomas can be divided into two categories: those in which colonic polyps are a significant feature of the disease (<1%) and where patients inherit a mutated copy of the APC gene, and those without this feature. Among the dominant syndromes associated with polyposis are hereditary non-polyposis colorectal cancer (HNPCC) (Lynch Syndrome I) and cancer family syndrome (Lynch Syndrome II) (1-3%). These are characterized by a defective DNA mismatch repair (MMR) system, which results in MSI (12). Although rare, these syndromes provide important insights into the biology of all types of CRC. The third and least understood pattern of colorectal carcinoma (CRC) development is familial CRC. Affected families exhibit a frequency of CRC higher than expected in a sporadic setting. It is estimated that approximately one-quarter of CRC cases fall into this category (13).

Multistep carcinogenesis mechanisms in colorectal cancer

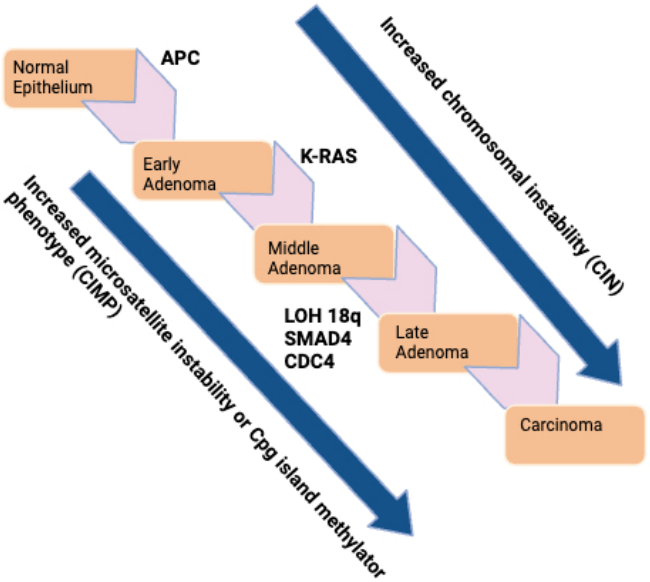

Figure 2. Multistep carcinogenesis mechanisms in colorectal cancer

In 1990, Fearon and Vogelstein developed the genetic paradigm for the development of colorectal cancer (CRC) (14). This multi-step genetic model suggests that the accumulation of various genetic and epigenetic changes in critical genes involved in the activation of oncogenes and the silencing of tumor suppressor genes (TSGs) is responsible for the development of CRC (15). In this context, two main pathways have been identified for the formation of CRC: the synthesis of APC and TSGs (14,16). The pathophysiology of CRC and its genetic instability are commonly associated with three pathways: microsatellite instability (MSI), chromosomal instability (CIN), and the CpG island methylator phenotype (CIMP) pathways (17).

The multi-step genetic model (Figure 3) indicates that the first stage involves the silencing of APC, followed by oncogenic KRAS mutations in the adenoma stage, and the occurrence of chromosomal 18q deletions along with TP53 inactivation during progression to malignancy. Additionally, genetic changes in TGF-βR and PI3KCA have been identified as contributing to the adenocarcinoma sequence model. Other genetic changes arising from metastatic lesions may include further genetic modifications, such as heterozygous loss (LOH) at 10q or increases in DNA sequences at 5p and 6p (17,18).

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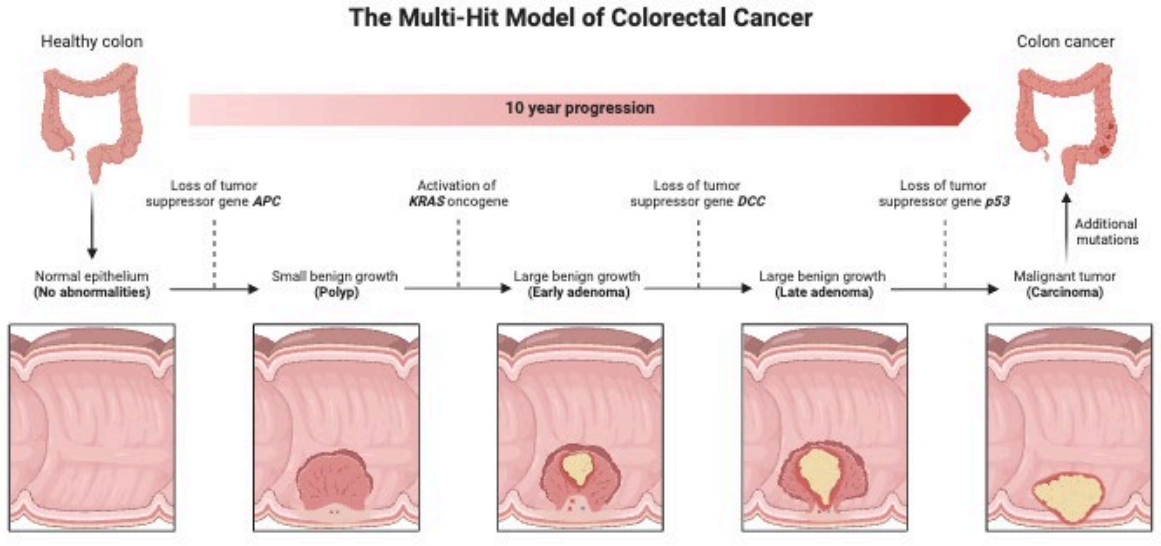

Figure 3. Stages of colorectal cancer development

The MSI status of colorectal cancers can be categorized as microsatellite stable (MSS; absence of a specific marker), MSI-low (MSI-L; presence of instability in one Bethesda marker), or MSI-high (MSI-H; presence of instability in at least two Bethesda markers). It has been reported that APC, KRAS, and TP53 are mutated in human MSI-H colorectal cancer and cancer cell lines containing MSI-H. BRAFV600E mutations are frequently observed in MSI-H colorectal cancers, but APC and TP53 mutations are less common (22,23). In Lynch syndrome types, activation mutations in BRAF are rare in colorectal cancer, but they are found in 5-10% of metastatic cases. Additionally, BRAF mutations were found in 4% and 40% of MSI-L and MSI-H tumors, respectively. The V600E (Val600Glu) hotspot mutation, commonly found in most BRAF mutations, is associated with a poor prognosis in individuals with colorectal cancer (24). Anti-EGFR antibodies, such as Cetuximab and Panitumumab, are ineffective against colorectal cancer with mutated KRAS (Mudd, 2018).

The CpG island methylator phenotype (CIMP) is a state of epigenomic instability characterized by the hypermethylation of multiple CpG islands. Tumor suppressor genes with hypermethylation in their promoter regions can lead to the loss of function of a gene in CIMP-positive cancers. CIMP-positive colorectal cancers grow along a convoluted pathway. In colorectal cancer, it has been shown that many genes are both methylated and silenced. APC, MLH1, MGMT, SFRP1, SFRP2, CDKN2A, TIMP3, VIM, SEPT, CDH1, and HLTF are among the frequently methylated genes. Furthermore, a unique subset of colorectal cancer, defined as having the CpG island methylator phenotype (CIMP), is characterized by frequent BRAFV600E mutations in CIMP tumors. PTEN, as a tumor suppressor gene, has low methylation levels, and promoter methylation in colorectal cancer silences the TWIST1 gene (27–29).

Molecular and biological foundations of colorectal cancer

Whether familial or sporadic, the development process from adenoma to cancer in colorectal cancer (CRC) requires a series of genetic changes. The accumulation of genetic and epigenetic changes leads to the transformation of normal cells in the mucosa into invasive adenocarcinoma. Genetic and epigenetic events related to CRC are studied under three main categories: Chromosomal Instability (CIN), Microsatellite Instability (MSI), and CpG Methylations.

Chromosomal instability (CIN)

Chromosomal Instability (CIN) is the most common form of genetic instability observed in patients diagnosed with colorectal cancer (CRC). CIN tumors are traditionally classified into two subgroups based on the frequency of chromosomal abnormalities: CIN-high (CIN-H) and CIN-low (CIN-L) tumors. The etiology of CIN is believed to originate from a disruption in the regulation of DNA replication checkpoints and processes controlling chromosome separation during mitosis, particularly at the mitotic spindle checkpoint (30). Compared to other forms of instability (MSI, CIMP), CIN is the most common feature in colon adenocarcinomas (present in 65-85% of cases) (31). In normal human cells, the average rate of

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genomic mutations is approximately 2.5 x 10^8 mutations per nucleotide per generation. However, this rate is higher in cancer cells due to the accumulation of multiple mutations during cell divisions (32,33). These changes predominantly involve DNA repair genes, oncogenes, proto-oncogenes (such as KRAS, c-Src, c-Myc activation), and tumor suppressor genes (TSGs) like TP53 and APC, as well as apoptotic genes. The pathogenesis through gene alterations requires at least seven different mutations to emerge, as mentioned above. The theory that CIN is pathogenic in CRC is supported by the discovery of significant chromosomal gains and losses during the adenoma-carcinoma sequence (e.g., loss of tumor suppressor gene p53 (TP53) and loss of heterozygosity (LOH) at the long arm of chromosome 18 (18q LOH)), indicating that CIN is an early event in tumor formation that increases as the tumor progresses (34).

CIN can be studied through various techniques, including karyotype analysis, DNA content flow cytometry (FCM), interphase fluorescence in situ hybridization (FISH), and comparative genomic hybridization (CGH) to detect DNA gain/loss. These techniques allow the detection of both numerical and structural chromosomal abnormalities in preneoplastic lesions (35).

CIN tumors are characterized by mutations in various TSGs: APC (up to 85%), TP53 (40-50%), SMAD2/4 (10-20%), and DCC (5%), as well as proto-oncogenes: KRAS (30-50%), CTNNB1 (5-15%), and PIK3CA (20%). In addition to studying proto-oncogenes and TSGs, amplifications of RB1, MYC, CCDN1, CCNE1, ERRB2 (HER2 gene), IGF2 (Insulin-like Growth Factor 2 Receptor gene), translocation of TCF7L1 (transcription factor 7-like 1 gene), and fusion of NAV2 (Neuron Navigator 2 gene) have been suggested to contribute to CIN, affecting cell cycle progression and mitotic control (7).

Microsatellite instability (MSI)

Microsatellite instability (MSI) is involved in the development of 15-20% of colorectal cancers (CRC) (36). MSI is associated with microsatellite repeats, which are present in nearly half a million locations throughout the human genome. Microsatellites are repetitive DNA sequences consisting of one to four base pairs, dispersed across both coding and non-coding regions of the genome. Due to their repetitive nature, microsatellites are prone to replication errors caused by the slippage of the DNA polymerase enzyme during DNA replication. If these replication errors are not corrected, they can lead to frameshift mutations or protein truncations. Under normal conditions, these errors can be repaired by the Mismatch Repair (MMR) system.

The MMR system consists of a protein complex made up of MLH1, MSH2, MSH6, PMS1, and PMS2. The inactivation of any of these proteins through mutation results in the tumor being categorized as MSI-positive (34,36). Microsatellite unstable tumors are more frequently located on the right side and tend to be poorly differentiated. They often display unusual histological types (mucinous) and significant peri-tumoral and intra-tumoral lymphocytic infiltration (37).

Microsatellite instability is also observed in patients with ulcerative colitis and is quite common in premalignant dysplastic and malignant lesions (21% and 19%, respectively). Lynch syndrome (formerly known as hereditary non-polyposis colorectal cancer) results from mutations in one of several MMR genes (MLH1, MSH2, PMS2, and MSH6). Changes in MMR genes or Polymerase ε (POLE) are also observed in sporadic cancers due to the adenoma-carcinoma transition associated with CIN. This transition takes 3-5 years, compared to approximately 20 years in sporadic CRC (38).

The Bethesda panel, commonly known as the standard test for MSI, was introduced by the National Cancer Institute (NCI) in 1997. This panel consists of five microsatellite loci: two mononucleotide repeats (BAT25, BAT26) and three dinucleotide repeats (D2S123, D5S346, D17S250). Studies using this panel categorize instability as low-level MSI (MSI-L) if instability is observed in only one of the five Bethesda panels (< 30-40%) or as high-level MSI (MSI-H) if instability is observed in all five panels (> 30-40%) (39). Tumors with no microsatellite instability detected in any locus are categorized as microsatellite stable (MSS). According to Centelles' studies, most MSI-L tumors do not exhibit MMR deficiency, whereas nearly all MSI-H tumors show MMR deficiency (40). In response to the need for more precise findings, Suraweera and colleagues introduced a new five-marker panel for MSI screening, which includes the mononucleotide repeats BAT25, BAT26, NR21, NR22, and NR24 (40).

For CRC patients with microsatellite instability, the standard treatment is 5-fluorouracil (5-FU). Recent studies suggest that MSI, particularly when significant deletions are present in HSP110, indicates a positive response to 5-FU treatment in combination with other drugs (41). The National Comprehensive Cancer Network (NCCN) recommends 5-FU, either alone or in combination with other drugs, for patients with stage II, III, and IV CRC, especially when the disease is associated with a poor prognosis (31).

CpG Island Methylator Phenotype (CIMP)

Another molecular disorder identified in CRC is CpG island (CGI) methylation. Independent of CIN and MSI mechanisms, hypermethylation of CpG islands in gene promoters is found in approximately 15% of CRC cases and

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leads to the epigenetic silencing of nearby genes (42). CpG islands are short sequences rich in CpG dinucleotides and can be found in the 5' region of about half of all human genes (43,44). DNA methylation is a common phenomenon that regulates gene expression and determines the structure of the cell nucleus. About 70% of CpG dinucleotides (cytosines preceding guanines in the DNA sequence) are methylated, meaning a methyl group is added to the cytosine (45). Compared to normal cells, tumor cells commonly show hypomethylation, which may be associated with chromosomal instability. They are often retained in an unmethylated state and are commonly found in the promoter regions of genes (46). Some of these CpG islands undergo hypermethylation in tumors, leading to transcriptional silencing and gene expression suppression (47). The transcriptional regulation of genes is directly influenced by the acetylation and methylation of histones, proteins that wrap DNA. Histone acetylation typically corresponds to transcriptional activation, whereas deacetylation generally corresponds to transcriptional inhibition (8).

The CpG island methylator phenotype (CIMP) is a variation of the normal DNA methylation pattern that leads to the universal dysregulation of gene expression associated with cell differentiation (48). Abnormal DNA methylation of CpG islands has been commonly observed in human colorectal tumors, and when this methylation occurs in promoter regions, it is associated with gene silencing (49). DNA methyltransferases (DNMT) carry out the enzymatic process of DNA methylation by adding a methyl group to the 5' position of cytosine, creating 5-methylcytosine. Abnormal methylation of CpG islands has been detected in genetic diseases such as Fragile X syndrome, in aging cells, and in neoplasia (50,51). Approximately half of the tumor suppressor genes that are mutated in patients with familial cancer syndromes, including Rb, VHL, p16, hMLH1, and BRCA1, have also been shown to undergo abnormal methylation in some sporadic cancers (52).

In some studies, promoter hypermethylation of multiple genes has been linked to poor prognosis in CRC, while other studies have reported better prognosis or no significant association (53,54). Poor prognosis in CIMP-high CRC patients may be due to factors closely related to CIMP, such as the BRAF V600E mutation, rather than the phenotype itself (53,55–57). MSI, which is strongly associated with CIMP, is another important candidate. However, MSI generally predicts a better prognosis. Additionally, patients with multiple promoter hypermethylations have been associated with shorter survival, often linked to the microsatellite stable (MSS) subgroup (53,58–60).

Signal pathways responsible for carcinogenesis in colorectal cancer

Genetic changes affect pathways related to WNT signaling, such as genes like APC, Axin 2, CTNNB1, and other pathways involving genes associated with RAS, PI3K, TGFβRII, SMAD2, SMAD4, and p53. Most CRC development occurs due to the dysregulation of several signaling pathways listed above.

The WNT Signaling Pathway in Colorectal Cancer Carcinogenesis

Activation of the canonical Wnt pathway is responsible for the initiation and progression of more than 90% of sporadic CRC cases. Wnt regulates various normal cell functions, such as embryonic development, cell polarity, cell proliferation, and fate determination. In normal cells, free β-catenin interacts with a complex containing APC, Axin, and glycogen synthase kinase-3β (GSK-3β), which ubiquitinates and destroys β-catenin (61). In CRC, genetic abnormalities in Wnt/β-catenin signaling components, such as APC, the β-catenin-coding gene CTNNB1, and Axin2, are typically responsible (62). APC mutations are often the initiating factor in colorectal adenomas and subsequent carcinogenesis. When the tumor suppressor gene APC fails to function properly, β-catenin accumulates, is transported to the nucleus, and initiates proliferative expression programs. In addition to mutations, APC deficiency can also be caused by other factors, such as hypermethylation of the APC promoter. This occurs in 18% of primary CRCs and adenomas (63).

As shown in Figure 4, the function of Wnt signaling is dependent on the amount of β-catenin in the cytoplasm. β-catenin can be degraded through phosphorylation and ubiquitination (64). These processes are carried out by a destruction complex consisting of core proteins such as AXIN, APC, casein kinase 1 (CK1), and glycogen synthase kinase 3 (GSK3). The two receptor molecules to which the Wnt ligand binds to initiate the signaling pathways are Frizzled proteins and low-density lipoprotein receptor-related proteins 5 or 6 (LRP5 or LRP6) (65). There are 19 Wnt interacting proteins and 10 Frizzled receptor members that can initiate either canonical or non-canonical Wnt signaling. Fzd7 plays a critical role in the initiation and spread of CRC. In addition to mutations in APC or CTNNB1, the Fzd7 protein is highly expressed in various colon cancer cell lines and tissues (66).

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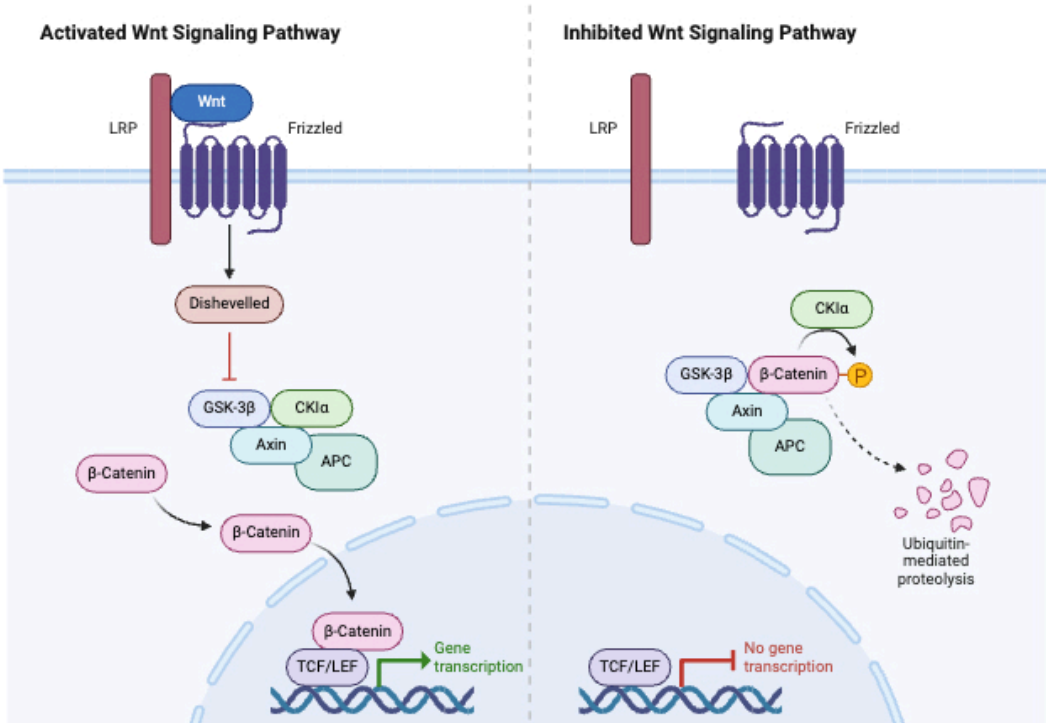

Figure 4. Activation and inhibition of the Wnt/β-catenin pathway

According to Herbergs and colleagues, patients with higher Fzd7 expression have a shorter overall survival and significantly higher Fzd7 mRNA levels in their tumors compared to normal tissues. Frizzled receptors respond to Wnt proteins only when the canonical β-catenin pathway is active, through the activation of LRP5 or LRP6 (67,68).

When Wnt proteins are unable to bind to their receptors, a "destruction complex" is formed consisting of components such as APC, Axin2 (adenomatous polyposis coli tumor suppressor), GSK3β, and casein kinase 1 (CK1). The phosphorylation of β-catenin, particularly at serine-675, by CK1 and GSK3β triggers the development of this "destruction complex." The β-TrCP (β-transducin repeat-containing protein), an F-box component of the E3 ubiquitin ligase complex, recognizes phosphorylated β-catenin and facilitates its ubiquitination, followed by degradation by the ubiquitin-proteasome system (69).

The development of cancer treatments has focused on downstream mediators such as the protein kinase (TNIK), which interacts with the catalytic inactive region of the tyrosine kinase adaptor protein-NCK. This is an important regulator of the TCF4/β-catenin complex and another downstream mediator like the tumor necrosis factor receptor-associated factor (TRAF). In preclinical models of colorectal cancer (KRK), TNIK inhibitors such as NCB0846 and FDA-approved Mebendazole targeting TNIK have been effective. This demonstrates the therapeutic potential of agents targeting Wnt pathways in KRK treatment. IWR-1, a tankyrase inhibitor, has potential in focusing on this point by modulating AXIN's PARylation. AXIN's PARylation is a key part of the Wnt signalosome and the β-catenin destruction complex. Other modulators of canonical Wnt signaling, including Niklosamide, which reduces the expression of Dv1-2 and β-catenin, are currently in clinical trials for KRK (70–73).

The TGF-β signaling pathway in colorectal cancer carcinogenesis

The TGFβ family plays a role in regulating various cellular processes. The tumor-suppressive function of TGF-β has been demonstrated in normal intestinal epithelium. Tumor-suppressive proteins like TGF-β are often lost in early and advanced stages of colorectal cancer (KRK) (74). In KRK, the growth inhibitory effects induced by TGF-β are ineffective

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(75). However, research has shown that TGF-β is highly active in the later stages of KRK formation. It triggers the production of various growth factors that promote cell growth and essentially accelerate tumor progression. Inside the cell, the main mediators of TGF-β signaling, SMAD proteins, interact with TGF-β receptors to become activated and migrate to the nucleus. In the nucleus, they regulate the transcription of certain genes critical for cancer progression (76). Two types of receptors have been identified: TβRII (which can bind TGF-β independently) and TβRI (which has a glycine/serine-rich region). When TGF-β binds to TβRII, it induces the recruitment of TβRI to the nucleus, and the glycine/serine-rich region is phosphorylated. Phosphorylated TβRI then collects and phosphorylates a gene regulatory protein (77). SMAD, when dephosphorylated, adopts a folded structure and loses its ability to bind DNA. However, upon phosphorylation, it opens up and forms dimers with additional SMAD molecules. This facilitates their migration to the nucleus, where they collaborate with other gene regulatory proteins to control the transcription of multiple genes. Ligand binding to TGF-β receptors also triggers the activation of non-SMAD signaling pathways, such as MAPK, PI3K, Notch, and Wnt signaling pathways, playing a role in activating various kinase cascades (78).

When 128 patients with metastatic colorectal cancer (mCRC) were examined using next-generation sequencing (NGS), alterations in the TGF-β pathways were identified in 17% of the metastatic colorectal cancer tissues (79). Additionally, genes regulated by abnormal DNA methylation, based on DNA methylation data downloaded from the Gene Expression Omnibus databases (GSE90709, GSE77955), demonstrated enrichment in the TGF-β signaling pathway (80,81). Since the transduction of traditional TGF-β signaling facilitates metastasis, alterations in SMAD proteins are crucial in the late stages of colorectal cancer (CRC). Patients with CRC exhibiting low SMAD activity are at a higher risk for lymph node metastasis (82).

Exome capture analysis of 224 individuals, both with and without malignancies, revealed that the SMAD4 and TGFBR2 genes are the most frequently altered. The mutation frequency of TGFBR2 in hypermutated tumors (including those with high microsatellite instability, MSI-high) is 51%, while SMAD4 and SMAD2 mutations are present in 10% of non-hypermutated cancers (83). Based on numerous high-throughput analyses, SMAD4 is one of the most altered genes in mCRC and requires further investigation. Using targeted NGS sequencing, SMAD4 mutations were detected in 22.8% of 123 metastatic CRC patients without MSI-high (79). Similarly, another study involving 32 metastatic CRC patients reported a SMAD4 mutation rate of approximately 6% (84). SMAD4 mutation frequencies in primary and metastatic CRC were found to be similar (15% and 14%, respectively) (85). SMAD4 mutations and deletions identified via NGS are strongly associated with invasive preclinical features in CRC patients (86). Among 330 early-onset mCRC patients, SMAD4 displayed recurrent mutations, with TGF-β pathway abnormalities detected in 30% of these patients. SMAD7 is a critical negative regulator of the TGF-β signaling pathway. In metastatic CRC tissues, SMAD7 expression is significantly lower compared to non-tumor tissues (87). Animals injected with clones expressing SMAD7 exhibited higher levels of TGFBR2 expression, TGF-β secretion, SMAD2 phosphorylation, and nuclear accumulation, potentially contributing to liver metastases compared to control mice. In a CRC splenic injection model, ectopic SMAD7 expression in nude mice facilitated cancer metastasis to the liver (88).

The PI3K/Akt Signaling Pathway in Colorectal Cancer Carcinogenesis

PI3K is one of the pathways activated by EGFR signaling. PI3K serves as a downstream target of receptor tyrosine kinases (RTKs) such as the epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), and insulin-like growth factor-1 receptor (IGF1R). These receptors are activated upon binding to their respective ligands (89). The PI3K signaling pathway plays a significant role in the development of colorectal cancer (CRC), with mutations in the PI3KCA gene observed in 15–20% of CRC patients. Pyrosequencing analyses often reveal a higher prevalence of PI3KCA mutations compared to Sanger sequencing (76).

PI3K is a heterodimeric molecule composed of three classes (Class I–III) that are distinguished by their structural and functional differences. Class IA is the most affected type in human cancer. This class includes two subunits of PI3K: a regulatory subunit (p85) and a catalytic subunit (p110). An important point to note is that colorectal cancers with PI3KCA mutations exhibit molecular and clinical heterogeneity. An NGS analysis of 206 metastatic colorectal cancer patients revealed a heterogeneous structure in patients with exon 9 mutations and exon 20 mutations. Patients were divided into three categories: those with exon 9 mutations (82%), those with exon 20 mutations (58%), and those with both exon 9 and exon 20 mutations (90).

AKT, a serine/threonine protein kinase (Ser/Thr kinase), is an enzyme that mediates the effects of PI3K on tumor growth and progression. The phosphorylation of AKT has been associated with cell proliferation and inhibition of apoptosis in human colorectal cancers (CRCs). Another way to

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activate PI3K is by stimulating the activation of PI3K through RAS or extracellular factors via receptor tyrosine kinases (RTKs) (91). The inhibition of P110 by P85 is relieved when RTK binds to intracellular phosphotyrosine residues, thereby activating PI3K. Phosphatidylinositol 4,5-bisphosphate (PIP2), phosphorylated by activated PI3K, subsequently produces phosphatidylinositol 3,4,5-trisphosphate (PIP3) (91).

AKT controls downstream signaling targets, such as mTOR, which facilitates growth, metabolism, angiogenesis, and protein translation. The tumor suppressor protein phosphatase and tensin homolog (PTEN) inhibits the PI3K pathway by dephosphorylating PIP3. Following PIP3 production, AKT activation promotes both cell survival and proliferation. In CRC, PI3KCA mutations are associated with phosphorylated AKT expression (34,92). The PI3K signaling pathway has been reported to play a significant role in CRC development and progression (76).

Activation of PI3K signaling increases prostaglandin E2 production and cyclooxygenase-2 (COX-2) activity, which suppress apoptosis in colon cancer cells. Aspirin can inhibit the PI3K pathway, halting the growth of colon cancer cells and inducing apoptosis (93). In in vitro environments, aspirin has been shown to induce apoptosis more significantly in colon cancer cell lines with PI3KCA mutations compared to those with wild-type PI3KCA (34).

The NOTCH signaling pathway in colorectal cancer carcinogenesis

The Notch signaling pathway has a tumor-promoting function but acts as a tumor suppressor in some cases. It has been shown that Notch signaling is essential for maintaining healthy intestinal epithelium. In the primary stages of colorectal cancer (CRC), Notch expression has been reported to be relatively higher compared to later stages. The Notch signaling pathway has at least five ligands: Jagged-1, Jagged-2, Delta-like-1, Delta-like-3, and Delta-like-4, and four receptors: Notch-1, Notch-2, Notch-3, and Notch-4. Additionally, the Notch signaling pathway is known to include several downstream target genes, such as p21, Hes-1, and Deltex (94–96).

Abnormal activation of Notch1 has been shown to trigger CRC diagnosed at various tumor stages. Gene sequence analysis has revealed that Notch1 and its target HES1 are significantly higher in advanced tumors compared to low-grade tumors (94). Fre et al. demonstrated the necessity of regular Wnt signaling for the proliferative effects of Notch signaling in early intestinal cancer precursors (95,97,98).

Fazio et al. linked Notch1 activation in CRC cells to the overexpression of matrix metalloproteinase-9 (MMP-9). The same study showed that increased AKT overexpression and Notch1 signaling result in enhanced CRC cell proliferation and an increase in tumor burden (99).

Epithelial-mesenchymal transition (EMT) has been proposed to result from interactions between Notch receptors and related ligands in CRC. Notch1 controls Jagged-1 activity, which in turn triggers Notch3 and increases CD44 and SLUG expression (95). In CRC, this condition leads to the EMT and stem cell-like properties.

Specific monoclonal antibodies (604,107 and 604,164) have been developed to target the Notch1 signaling pathway in breast and colon cancer cell lines. Combining monoclonal antibodies specific for these mutants with doxorubicin inhibited the growth of xenografts derived from breast and colon cancer cells and increased tumor regression (100). In vitro studies have found that CRC cells with increased HES1 expression are more resistant to 5-FU treatment. Furthermore, increased HES1 expression has been associated with poor prognosis in CRC (101,102). HES1 controls the invasive ability of CRC cells via the STAT3-MMP14 pathway. In individuals with stage II CRC, a high correlation was found between HES1 expression and disease relapses after treatment. HES1 inhibitors such as crenigacestat (LY3039478) have shown therapeutic efficacy in CRC patients (95).

In Table 1 mutations observed in colorectal cancer and their associated signaling pathways, along with corresponding clinical therapies are summarized.

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Table 1. Mutations and associated signaling pathways in colorectal cancer

GENES	Type of mutation	Affected signaling pathways	Tumor suppressor gene/Onco gene	Treatment approach	References
PLA2G2A	Somatic	The activity of the Wnt-beta catenin signaling pathway can be suppressed, leading to the release of arachidonic acid, which promotes cancer development by increasing the production of inflammatory mediators.	Tumor suppressor gene	-	(103,104)
NRAS	Somatic	It promotes cell proliferation through ERK1/2 activation via the Ras-MAPK signaling pathway and stimulates AKT phosphorylation by increasing the activity of PI3K.	Oncogene	Cetuximab (Erbitux) Panitimumab (Vectibix)	(105–107)
BUB1	Somatic /Hereditary	It functions as a mitotic checkpoint protein and ensures the proper separation of chromosomes. A dysfunction in its activity leads to aneuploidy.	Tumor suppressor gene	-	(108,109)
CTNNB1	Somatic	It is a key component of the Wnt/β-catenin signaling pathway. It regulates mechanisms of cell adhesion, particularly controlling cell proliferation. The effects of the Wnt/β-catenin signaling pathway also influence the PI3K/AKT signaling pathway, the stem cell signaling pathway (NOTCH), and the TGF-beta signaling pathways.	Oncogene	Foscenvivint (PRI-724) (clinical trials)	(110–113)

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PIK3CA	Somatic	Mutant PIK3CA activates AKT signaling, which upregulates fatty acid synthase.	Oncogene	Buparlisib (BKM120) (114)	(115)
FGFR3	Somatic	The activation of the MAPK/ERK pathway can support cell proliferation, differentiation, and survival. Interaction with the PI3K/AKT signaling pathway can enhance the survival ability of tumor cells and contribute to the development of resistance to treatment. Activation of the STAT pathway promotes cell growth and survival. Differentiation of the PLCγ pathway can increase intracellular calcium levels.	Oncogene	Pemigatinib (FGFR Inhibitor) (116) Erdafitinib (117)	(118)
TLR2	Somatic	Activation of TLR2 activates the NF-κB signaling pathway. This activation leads to the release of inflammatory cytokines and other inflammatory mediators. Activation of the JAK/STAT pathway can cause immune cells to play a tumor-supporting role or allow cancer cells to suppress the immune response.	Receptor	-	(119–122)
APC	Somatic/ Hereditary	It is a negative regulator of the Wnt/β-catenin pathway. Loss of APC function leads to the invasiveness of cells and increases their ability to metastasize.	Tumor suppressor gene	Porcupine Inhibitors (LGK974) (123)	(124,125)
MCC	Somatic/ Hereditary	It is associated with the Wnt/β-catenin signaling pathway and regulates cell proliferation and differentiation.	Tumor suppressor gene	-	(126)
PTPN12	Somatic	It plays a role in altering RAS/MEK/ERK signaling.	Tumor suppressor gene	-	(127)
BRAF/BRAF V600E	Somatic/ Hereditary	Since it is part of the RAF–MEK–ERK1/2 pathway, mutations observed here mimic the biological consequences of KRAS mutations.	Oncogene	Vemurafenib, Dabrafenib, Encorafenib	(129–132)

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				(128)	
DLC1	Somatic	It plays a role in the regulation of Rho GTPases, thereby affecting cellular motility and invasion.	Tumor suppressor gene	Rho GTPaz Inhibitors	(133,134)
PDGFRL	Somatic	Through the PI3K/Akt, MAPK/ERK, and Rho GTPase signaling pathways, it can affect cell growth, proliferation, and invasion.	Tumor suppressor gene	-	(135,136)
RAD54B	Somatic	Homologous recombination plays a role in DNA repair through the ATM/ATR signaling pathway and the p53 signaling pathway.	Tumor suppressor gene	Olaparib (137)	(138)
PTPRJ	Somatic	EGFR, VEGFR, and Rho GTPase signaling pathways regulate cell proliferation, cell motility, angiogenesis, and cellular energy production through the Krebs cycle.	Tumor suppressor gene	EGFR Inhibitors (Cetuximab etc.) (139)	(140,141)
CCND1	Somatic	High levels of CCND1 can increase cell proliferation. Additionally, increased expression on the PI3K/Akt pathway leads to uncontrolled cell division, while the RAS/ERK pathway further enhances cell proliferation.	Oncogene	Lapatinib (EGFR/HER2 kinase Inhibitors) Palbociclib (142,143)	(144,145)
MLH3	Somatic	As part of the error repair pathway, it plays a critical role during DNA replication. It regulates cell proliferation through the Wnt/beta-catenin signaling pathway, while also demonstrating tumor-suppressive properties through the TGF-beta signaling pathway.	Tumor suppressor gene	-	(146,147)
AKT1	Somatic	It regulates cell proliferation through the PI3K/AKT signaling pathway, protein synthesis and cellular metabolism through the mTOR signaling pathway. By increasing the expression of factors such as VEGF, it also promotes angiogenesis.	Oncogene	Ipatasertib (148) MK-2206 (149) Everolimus (150)	(152–154)

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				Temsirolimus (151)	
BUB1B1	Somatic /Hereditary	It is critical for cell cycle regulation and proper chromosome segregation during mitosis.	Oncogene	-	(155,156)
TP53	Somatic /Hereditary	It regulates critical cellular processes such as the cell cycle, DNA repair, and apoptosis.	Tumor suppressor gene	-	(157–159)
FLCN	Somatic	In cancer cells, uncontrolled proliferation, angiogenesis, and increased metastasis are driven by the activation of mTOR, AMPK, Wnt/beta-catenin signaling pathways, and HIF-1α.	Tumor suppressor gene	-	(160,161)
AXIN2	Somatic /Hereditary	It is a negative regulator of the Wnt signaling pathway. Normally, a complex that includes AXIN2 facilitates the degradation of β-catenin. However, mutations in the AXIN2 gene can lead to the accumulation of β-catenin within the cell, which then translocates to the nucleus, activating Wnt target genes.	Tumor suppressor gene		(162,163)
DCC	Somatic	By interacting with the Netrin-1 ligand, it regulates processes such as adhesion. In the absence of Netrin-1, it can initiate apoptosis signaling. Since it regulates intercellular interactions in the tumor microenvironment (TME), loss of DCC can lead to increased inflammation and, through the PI3K/AKT and MAPK/ERK signaling pathways, enhance cell proliferation.	Tumor suppressor gene	-	(164–166)
BAX	Somatic /Hereditary	It promotes intrinsic (mitochondrial) apoptosis.	Tumor suppressor gene	-	(167,168)
SRC	Somatic	It is involved in the activation of the NF-κB and PI-3K/Src signaling pathways.	Oncogene	Dasatinib (169–171) Saratinib(172, 173)	(174)

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AURKA	Somatic /Hereditary	It is involved in the regulation of Wnt and RAS-MAPK signaling genes.	Oncogene	-	(175)
EP300	Somatic	Epithelial-mesenchymal transition (EMT) plays a role in active angiogenesis and matrix remodeling.	Oncogene	-	(176–178)
SMAD2/SMAD4		It is a mediator of the TGF-β signaling pathway.	Tumor suppressor gene	-	(179)

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Mutations in signaling pathway components

KRAS plays an important regulatory role in cell signaling pathways such as the PI3K-Akt and RAS-RAF-MAPK pathways, which are involved in cell proliferation, as well as the RAS-Guanine nucleotide exchange factor (GEF) signaling pathway associated with cytokine production. KRAS mutations, one of the earliest mutations leading to CRC (colorectal cancer), account for about 40% of cases (180). Approximately 90% of KRAS mutations typically occur at codons 12 and 13 in exon 2. Codons 59, 61 (in exon 3), and 117, 146 (in exon 4) are less commonly affected by these mutations (181,182). CRCs with KRAS mutations in exons 3–4 are more frequently associated with mucinous/rare histological subtypes. KRAS-mutated CRCs are typically well/moderately differentiated tumors, usually linked with the classical adenocarcinoma subtype, and commonly exhibit a microsatellite stable phenotype (183). In cases where KRAS activation is not the initiating event, Boutin et al. demonstrated the necessity of KRAS expression for tumor maintenance in an experimental mouse model, where mutations in APC, P53, and KRAS are spatially and temporally regulated (34,184).

Treatment strategies include inhibiting downstream signaling molecules such as RAF and MEK, which are associated with KRAS. RAF is a direct downstream effector of KRAS. Targeted inhibition of RAF has become the first choice to block KRAS signaling. However, RAF inhibition activates MEK through a feedback loop. Therefore, directly inhibiting RAF to achieve good clinical outcomes in KRAS mutant CRC treatment is difficult (185). A MEK 1/2 inhibitor, Selumetinib (AZD6244), is designed to inhibit the MEK enzyme in the RAS/MAPK pathway (186). Trametinib is a potent, selective, ATP-competitive inhibitor of MEK1/2 kinases. Another MEK inhibitor, GDC-0623, is currently being studied in a Phase I clinical trial to upregulate BIM expression (187). Targeting metabolic processes may be a therapeutic approach in KRAS-mutant CRC, as KRAS mutations affect cancer metabolism that supports disease growth. Mutant BRAF or KRAS causes cancer cells to overexpress GLUT1, which encodes the glucose transporter-1 that plays a key role in glucose metabolism (188). KRAS mutant CRC cells can withstand low glucose environments and exhibit enhanced glucose uptake and glycolysis. In a different study, the glycolysis inhibitor 3-bromopyruvate selectively inhibited the proliferation of cancer cells carrying BRAF or KRAS mutations (189).

Among all mutations in metastatic CRC, BRAF gene mutations account for 8-12% (190). BRAF mutations occur in the early stages of carcinogenesis and are considered oncogenic drivers as they lead to the formation of epithelial cells in serrated adenomas. Approximately 50 different BRAF mutations associated with CRC have been reported (191). However, BRAFV600E substitution mutations are believed to result from a series of sequential phosphorylations in gene transcription that activate a constitutive kinase capable of activating the RAS/RAF/MEK/ERK pathway, representing 80% of cases (192). BRAF V600E is associated with a variety of features, including mucinous and poorly differentiated histology, high CpG island methylator phenotype (CIMP) levels, and microsatellite instability (MSI-high). In individuals with BRAF V600E mutations, poor response to chemotherapy, short progression-free survival, low overall survival rates, and higher likelihood of developing distant lymph node metastases and peritoneal metastases are observed (193).

CRC is associated with EGFR modulation, including EGFR overexpression, EGFR mutations, gene amplification, and copy number alterations (194). Monoclonal antibodies (mAbs) have shown promising results in metastatic CRC treatment. Currently, two anti-EGFR mAbs have been approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for metastatic CRC treatment. The mechanisms of action of Cetuximab and Panitumumab are similar; they bind to the extracellular domain of EGFR, blocking the ligand-binding site, preventing the activation of tyrosine kinase, and leading to protein internalization and degradation. By blocking downstream EGFR pathways, they promote apoptosis (195). Cetuximab (ERBITUX), a chimeric (human-mouse) IgG1 monoclonal antibody, binds to the extracellular domain of EGFR with high affinity. Preclinical and clinical studies have demonstrated Cetuximab's ability to block the EGFR pathway. Preclinical studies have shown Cetuximab's cytostatic activity on its own, but when combined with other chemotherapeutic drugs such as irinotecan and platinum-based compounds, it has improved the antitumor activity of each regimen (195). According to Phase II studies, in patients with advanced CRC, Cetuximab alone showed an 11% response rate, while when combined with irinotecan, the response rate increased to 23% (196) Panitumumab, a fully humanized IgG2 mAb, has been used as a third-line treatment agent for metastatic CRC in the US and Europe.

In colorectal cancer, sensitivity to Hsp90 inhibitors, combinations of sorafenib and irinotecan, MEK and PI3K/mTOR inhibitors is associated with preclinical studies and linked to mutations in NRAS, KRAS, and "classical" BRAF genes. Similarly, the efficacy of Bcl-2/Bcl-xL and mTOR inhibitors, as well as the effects of sorafenib or MEK inhibitors, anti-EGFR drugs, and mTOR inhibitors, is being investigated. Ongoing research is focusing on KRAS and NRAS gene mutations as potential indicators of response to the anti-VEGF agent bevacizumab. Tumor cell resistance to vemurafenib and dabrafenib, but increased sensitivity to sorafenib (a multi-

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kinase inhibitor) and MEK inhibitors, may be linked to non-classical BRAF mutations (31).

A significant proportion of sporadic CRCs, ranging from 50% to 75%, either show allelic deletion of 17p or inactivating point mutations in the p53 gene. However, these genetic alterations are found in only a smaller subset of adenomas, with a frequency ranging from 4% to 26% (197,198). Therefore, mutations in the p53 gene generally occur in the later stages of the adenoma-carcinoma progression (199).

Conclusions

CRC is typically caused by a combination of genetic predisposition and environmental factors. The process from normal epithelium to carcinoma via a polyp involves various genetic changes, such as the activation of specific oncogenes and the inactivation of tumor suppressor genes, occurring over a period of approximately 8-12 years. Mutations in p53, APC, K-RAS, and/or changes in proteins such as the loss of APC and microsatellite instability or loss of heterozygosity are well known (200). Mutations can arise because of environmental stress in somatic cells or exist as hereditary germline abnormalities, both of which continue to contribute to colon tumor formation (201).

CRC has four main stages, and in addition, a fifth stage, known as "recurrent," has been described. For each stage, there are many treatment options available. The current standard approach for colon cancer involves 6 months of adjuvant 5-FU therapy combined with leucovorin, which significantly reduces recurrence rates and the risk of death from resected colon cancer. This regimen significantly increases the 3-year disease-free survival rate. However, only 26% of patients truly respond (202). An important issue limiting the effectiveness of chemotherapy is drug resistance. According to a recent study, human colon cancer cells resistant to 5-fluorouracil (FU) overexpress Bcl-XL, Bcl-Xs, and Bik proteins. Short interfering RNA specific to Bcl-XL was found to be more effective in inhibiting the proliferation of 5-FU-resistant cells when Bcl-XL protein expression was reduced (203).

Chronic use of traditional non-steroidal anti-inflammatory drugs (NSAIDs) has been associated with a reduced risk of colorectal cancer (204). Selekoksib, a selective COX-2 inhibitor, has been shown in recent clinical trials to be more gastrointestinal-safe than traditional NSAIDs while also being effective in reducing colorectal adenomas in individuals with FAP (familial adenomatous polyposis) and in animal models. The FDA has approved two COX-2 inhibitors, Selekoksib and Refokoksib, as adjunctive treatments for patients with FAP (204–206).

The immune system can be activated by tumor-associated antigens expressed by colon tumor cells (207). Monoclonal antibodies (mAbs) targeting tumor antigens have the ability to facilitate the elimination of tumor cells by opsonizing them and activating immune effectors such as the complement cascade or natural killer cells (208). In the treatment of micro-metastatic disease, the use of Edrecolomab, a murine monoclonal antibody recognizing the epithelial cell adhesion molecule Ep-CAM (monoclonal antibody 17-1A), has been shown to provide significant benefits (209). In a study involving 189 patients with resected stage III colorectal cancer, treatment with Edrecolomab resulted in a 32% increase in overall survival and a 23% reduction in tumor recurrence rate (201).

Avastin (Bevacizumab) was FDA-approved for the treatment of colon cancer patients in 2004 and is used for anti-angiogenic purposes. VEGF is a protein that Avastin aims to block. The mechanisms of colorectal carcinogenesis are increasingly being elucidated. By identifying new components involved in the development of CRC and considering the variables that emerge in the disease through an integrated approach, more ideal targeted therapies can be developed.

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Castleman disease of the parotid gland (hyaline vascular type): A case report

Parotis bezinin castleman hastalığı (Hiyalin VaskülerTip): Vaka sunumu

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Parotid Gland (Hyaline Vasculer Type)
Received: 18.12.2024
Accepted: 21.05.2025
Doi: 10.18614/dehm.1751168

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ABTSTRACT

Castleman Disease (CD) is a rare lymphoproliferative disease characterized by painless lymph node involvement. It is infrequent in the extrathoracic area. One of the areas where it is rarely seen is the salivary glands. In this study, the diagnosis and treatment of a 38-year-old patient with localized parotid CD were presented. The patient had a mass in the left parotid gland for about 6 years. Surgery was planned after the evaluation. Left superficial parotidectomy and left second zone neck exploration was performed. Macroscopic examination of the patient's pathology sample revealed several nodular gray tissue pieces and a large solid-looking, yellow and pink tissue piece. In the microscopic examination, in addition to the reactive lymph nodes in the left zone 2 of the neck, paracortical expansion clearly separated from the parotid gland structures in the main parotid mass, prominence in the mantle zone, follicular hyperplasia, fused germinal center structures (twinning) in some of the follicles, hyalinized sclerosis with transverse entry into some germinal centers. Vascular sections (lollipop image and twinning), vascular proliferation in the interfollicular area, significant hyalinization in the vessel walls, and lymphoid proliferation with increased plasma cells were observed. The described features were reported as compatible with "Hyaline vascular type Castleman Disease". There was no recurrence during the patient's 2.5-year follow-up period. CD is a disease diagnosed by histopathological examination. Surgical treatment is sufficient in patients with localized involvement. Patients with residual disease or multicentric cases may require more aggressive treatment.

KEYWORDS

Castleman disease, parotidectomy, salivary glands.

ÖZ

Castleman Hastalığı (CH), ağrısız lenf nodu tutulumu ile karakterize nadir bir lenfoproliferatif hastalıktır. Ekstratorasik bölgede nadirdir. Nadir görüldüğü alanlardan biri de tükürük bezleridir. Bu çalışmada, lokalize parotis CH'si olan 38 yaşındaki bir hastanın tanı ve tedavisi sunulmuştur. Hastanın yaklaşık 6 yıldır sol parotiste kitlesi vardı. Değerlendirme sonrasında cerrahi planlandı. Sol süperfisyal parotidektomi ve sol ikinci zon boyun eksplorasyonu yapıldı. Hastanın patoloji örneğinin makroskobik incelemesinde birkaç nodüler gri doku parçası ve solid görünümlü, sarı ve pembe renklerde büyük bir doku parçası gözlendi. Mikroskobik incelemede boynun sol 2. bölgesinde reaktif lenf nodlarına ek olarak ana parotis kitlesinde parotis bezi yapılarından belirgin olarak ayrılmış parakortikal genişleme, manto bölgesinde belirginleşme, foliküler hiperplazi, bazı foliküllerde kaynaşmış germinal merkez yapıları (ikizleşme), bazı germinal merkezlere transvers girişli hiyalinleşmiş skleroz görüldü. Vasküler kesitler (lolipop görüntüsü ve ikizleşme), interfoliküler alanda vasküler proliferasyon, damar duvarlarında belirgin hiyalinleşme ve plazma hücre artışı ile lenfoid proliferasyon gözlendi. Histopatolojik inceleme "Hiyalin vasküler tip Castleman Hastalığı" ile uyumlu olarak raporlandı. Hastanın 2,5 yıllık takibinde nüks gözlenmedi. Lokalize tutulumu olan hastalarda cerrahi tedavi yeterlidir. Rezidüel hastalığı olan veya multisentrik olgular daha agresif tedaviye ihtiyaç duyabilir.

ANAHTAR KELİMELER

Castleman hastalığı, parotidektomi, tükürük bezleri.

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Castleman Disease, also known as giant lymph node hyperplasia, angiofollicular hyperplasia, or lymphoid hamartoma, is a rare lymphoprolipherative disorder. There have been limited reported cases globally. Initially delineated in 1956 by Castleman, it manifests as a benign localized lymph node enlargement predominantly found in the mediastinum of asymptomatic individuals. The most commonly affected area is the mediastinum, it is infrequent in the extrathoracic area. One of the areas where it is rarely seen is the salivary glands (1).

Additional types have subsequently been identified that expand the spectrum of this heterogeneous group of diseases (1-2). There are two main pathological variations of CD. The hyaline-vascular variant is the most common (> 90%). It is characterized by small hyaline-vascular follicles and capillary proliferation. Another is the plasma cell variant (10%), in which large lymphoid follicles are separated by plasma cell sheets. Hyaline-vascular cases are usually asymptomatic. The plasma cell variant presents with polyclonal hypergamma-globulinemia as well as fever, anemia, weight loss, and night sweats. CD is most commonly seen (60%) in the mediastinum. It can also be foundseen in extrathoracic areas, such as the neck, armpits, mesentery, pelvis, pancreas, adrenal glands, and retroperitoneum. In 14% of cases, involvement in the head and neck region is observed, with 85% of these occurrences specifically localized to the neck region. Establishing a preoperative diagnosis is often extremely challenging and with research endeavours frequently yielding inconclusive results (2-6).

Case Presentation

A 38-year-old female presented with a painless swelling located on the left side of her face. The patient reported no other complaints or medical conditions aside from the swelling, which had persisted for approximately 6 years. On physical examination, a palpable, partially mobile, well-defined mass measuring approximately 4 cm was detected in the left parotid gland region. No additional palpable lymph nodes were noted in the neck. Facial nerve examination was normal. The patient underwent a comprehensive series of blood tests, including complete blood count, biochemistry panel, prothrombin time, partial thromboplastin time, and serological analysis,; all of the results were within the normal range.

Additionally, chest radiography and electrocardiography yielded normal findings. Subsequently, ultrasonography (USG) and computed tomography (CT) imaging were conducted. The USG and CT scans revealed a space-occupying lesion measuring 28 × 16 × 40 mm with hyperechoic foci located in the posterior region of the left parotid gland. There was also a 16x8 mm hypoechoic thick lymph node in the left level 2 of the neck. A 3 cm hyperdense mass was seen in the left parotid in the contrast-enhanced CT. In the retrospective examination of the patient, it was observed that the mass was 34x14 mm in size in the USG performed approximately 3 years ago. The fine needle aspiration biopsy (FNAB) performed at an external center reported no neoplasia. It was learned that the patient had been offered surgery at that time, but had declined.

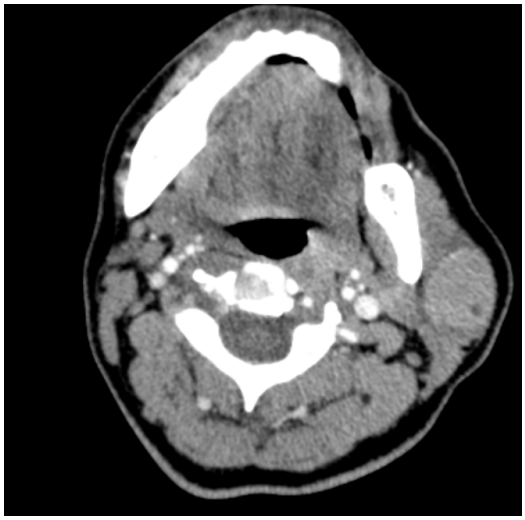

Figure 1. CT image of the mass in the left parotid

The patient was informed about the operation and agreed to undergo surgery. Left superficial parotidectomy and left second zone neck exploration was performed. It started with a Lazy S incision. Once the main trunk of the facial nerve had been located, the parotid gland mass was removed by tracing the branches of the nerve. Additionally, the left 2nd region of the neck was explored. A lymph node thought to be reactive was removed and included in the specimen.

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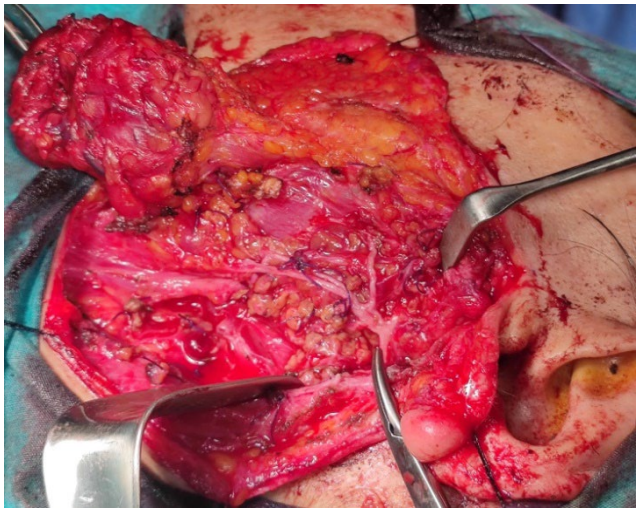

Figure 2. Mass in the left parotid and facial nerve branches

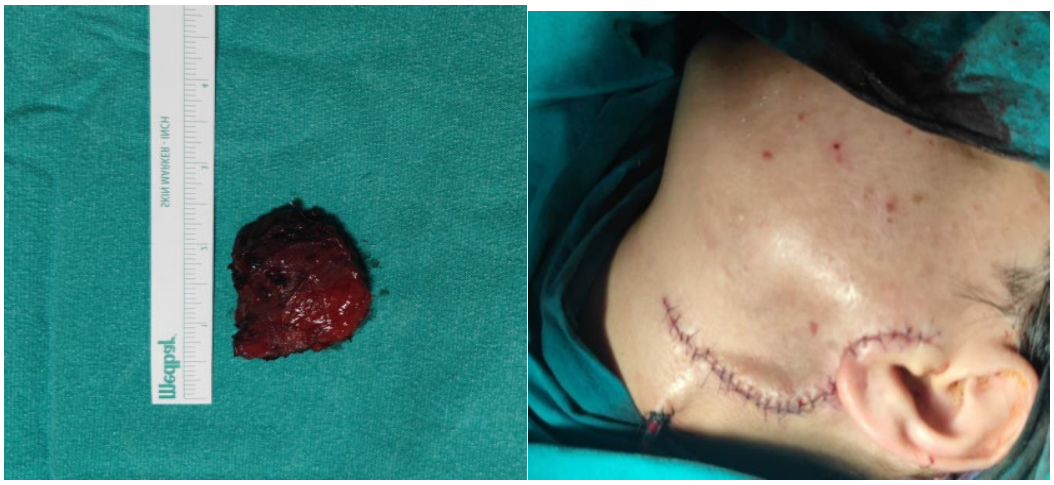

Figure 3. Mass image after removal and Lazy S incision—post-operative skin closure

In our case, the macroscopic examination of the patient's pathology specimen, revealed a few nodular grayish tissue pieces, as well as a large tissue piece with a solid appearance and yellow and pink colors, when a section of 4.5x4x1.7 cm in size was made. In the microscopic examination, in addition to the reactive lymph nodes in the left zone 2 of the neck, paracortical expansion clearly separated from the parotid gland structures in the main parotid mass (Figure 4), prominence in the mantle zone, follicular hyperplasia, fused germinal center structures (twinning) in some of the follicles, and hyalinized sclerosis with transverse entry into some germinal centers were demonstrated.

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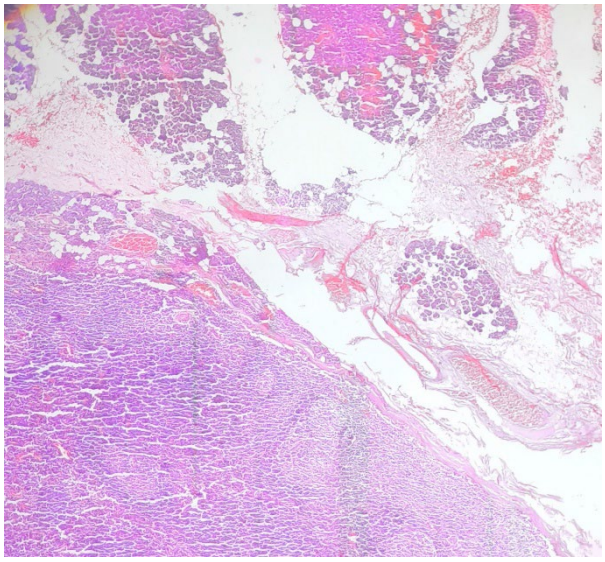

Figure 4. Preserved salivary gland structures are observed at the top, and lymphoid tissue related to the disease is observed at the bottom. H&E, x40

Vascular sections (Figure 5 - lollipop image and twinning), vascular proliferation in the interfollicular area, significant hyalinization in the vessel walls, and lymphoid proliferation were observed. In immunohistochemical study; positive staining was observed in the follicular dendritic network with CD21 and CD23. CD38 and CD138 staining revealed rare plasma cells in the germinal centers and interfollicular areas. Kappa and lambda light chains were positive in some of the plasma cells (polytypic). Diffuse plasma cell proliferation was not observed. Follicular lymphoma (CD10, BCL6, and BCL2) and mantle cell lymphoma (CD5 and Cyclin D1) were excluded based on immunohistochemical staining. The described features were reported as compatible with "Hyaline vascular type Castleman Disease".

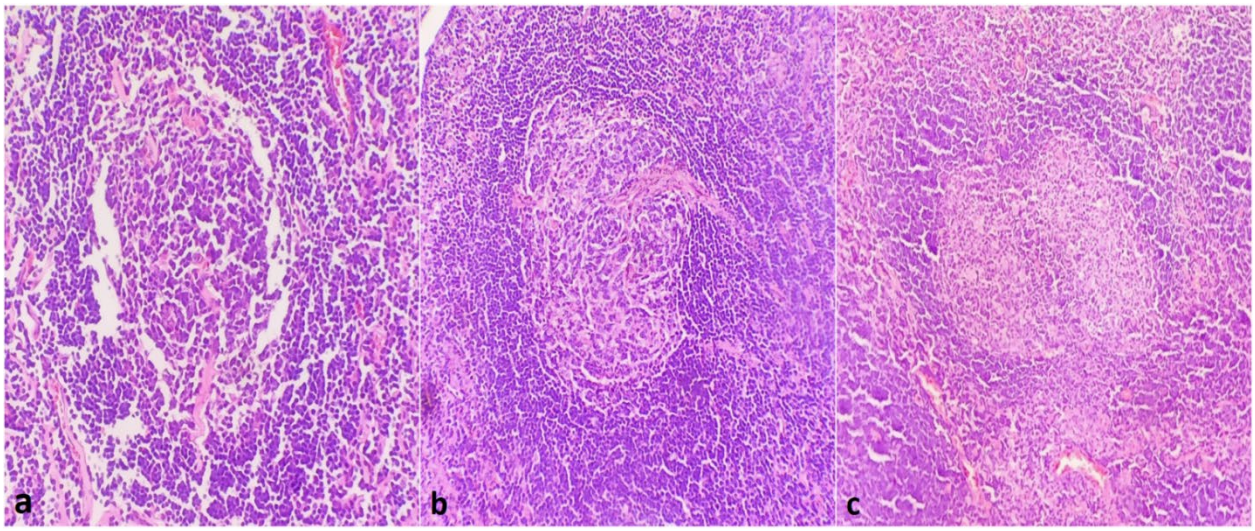

Figure 5. a,b. Lollipop follicle. Hyalinized sclerosing vessel sections with transverse entrance to the germinal center. c. Germinal center structures fused in follicles (twinning).

CD is a rare disease that is called giant lymph node hyperplasia, angiofollicular lymph node hyperplasia, angiomatous lymphoid hamartoma, and Castleman's lymphoma (7). The most commonly affected area is the mediastinum, it is infrequent in the extrathoracic area. One of the areas where it is rarely seen is the salivary glands. In a study, it is mentioned that 112 cases were reported involving the neck area. It is mentioned that 22 of these cases, including their own, involved the parotid gland (7). Pathogenesis is not clearly understood. Some authors argue that it is lymphoid hyperplasia resulting from an immunological response, while others say that it is caused by a benign tumor or hamartoma (8-10). The hyaline vascular variant is the most common subtype of CD. The hyaline vascular type is characterized by small lymphocytes that are arranged concentrically around it. The most common finding is multiple small follicle-like structures with marked vascular proliferation and hyalinization. It is usually localized, and has a favourable clinical course. Another, less common variant, is the plasma cell variant. It is

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characterized by mature plasma cell sheets in the interfollicular spaces and larger hyperplastic follicles with less vascular proliferation. This type is often associated with constitutional symptoms such as fever, fatigue, weight loss, and erythrocytosis. Plasma cell type variant requires close monitoring after surgery, and systemic chemotherapy may be required (11-14)

This disease is difficult to diagnose because it is rare and has no typical signs or symptoms. There are few definitive radiological findings, and lesions tend to mimic other head and neck sites..FNAB is not diagnostic. Definitive diagnosis of CD depends on histopathological examination (5,8,14-17). Although CD has been diagnosed with fine needle aspiration cytology in a few publications, a reliable preoperative cytological diagnosis is generally not possible in routine practice (18,19) It can be confused with lymphoid malignancies, such as mantle cell lymphoma and follicular lymphoma, as well as lymphadenopathies caused by non-neoplastic conditions, including systemic lupus erythematosus, rheumatoid arthritis, and IgG4-related disease. The reliability of fine needle aspiration cytology in the initial diagnosis of lymphoma is controversial, and the diagnosis can be further supported by additional studies, such as flow cytometry (20, 21).

Immunohistochemical studies are crucial for distinguishing CD from low-grade lymphomas. While immunohistochemical stains can be applied to cell blocks obtained from cytological samples, histological sections provide more reliable results. In CD, CD20-positive atretic germinal centers are observed, while the interfollicular areas are rich in CD3- and CD5-positive T lymphocytes. Immunohistochemistry (IHC) can also be used to highlight residual germinal centers (BCL-6,CD10) and follicular dendritic cell networks (CD21). CD138-positive polytypic plasma cells in the interfollicular areas are used to distinguish between hyaline vascular and the plasma cell types of CD (22, 23).

In most patients with parotid gland CD, the lesions are localized (17). Patients with localized CD are treated with complete surgical excision, and recurrence is rare. Radiotherapy may be considered in patients with residual disease. Additionally, more aggressive treatment may be required in multicentric cases. Chemotherapy may be recommended in addition to surgery (5, 8, 11, 24-26). In our case, the patient was also referred to the hematology department after the operation. The patient's disease was accepted as localized parotid gland CD and follow-up was recommended by hematology. No recurrence was observed in the patient's follow-up neck and parotid gland ultrasounds, who was followed for approximately 2.5 years.

In conclusion, CD is a benign lymphoproliferative disease. It is not frequently located in the extrathoracic area. While its location in the head and neck region is rare, salivary gland involvement in this area is even rarer. CD should be kept in mind in the differential diagnosis of salivary gland tumors. While surgical excision is the preferred treatment for single-center CD, additional treatments are required for residual cases of multi-centre CD. Good cooperation between ENT, pathology, and haematology is essential for diagnosing, treating and monitoring this disease.

Author contribution

Gülten Benan Göçer, Mustafa Nacir, Ali Mızrak Data curation, Formal analysis, Methodology, Validation. Gülten Benan Göçer Writing: review & editing.

Declarations

The authors declare no conflict of interest.

Declaration of ethical code

The authors declares that the materials and methods used in this study do not require ethical committee approval or legal-specific permission

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Complete Book: Ravel R. Clinical Laboratory Medicine. Fourth Edition. Chicago: Yearbook Medical Publishers Inc, 1984; 265-281.

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exercise testing. Safety and performance guidelines. Medl Aust 1996; 164-228.

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