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Research Article
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DETERMINATION OF NITROSAMINES IN VARIOUS PHARMACEUTICALS AT VARIABLE TEMPERATURES

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Abstract: Nitrosamines have been classified as potent genotoxic agents for humans by the International Agency for Research on Cancer. Our study aimed to determine the levels of nitrosamine impurities that could be formed in the content of drugs under different temperature conditions during their shelf life using chromatographic analysis. Eleven drugs in pharmacies were subjected to long-term exposure at two different temperatures. Twelve nitrosamine impurities of all samples were performed using LC-MS/MS. When the impurity levels of the analyzed drugs were examined, no nitrosamine impurity was detected in any drugs. Our study revealed that if no impurity was detected under storage conditions, there was no impurity formation even when the temperature was increased. When impurity formation is effectively prevented during the manufacturing stage, the risk of impurity occurrence during the shelf-life of drugs belonging to the same group is estimated to be low.

Keywords: Drug, Impurity, LC-MS/MS, Nitrosamine, Toxicology

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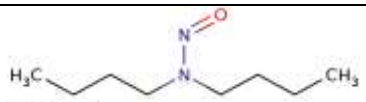
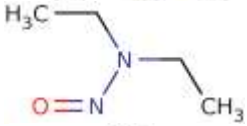
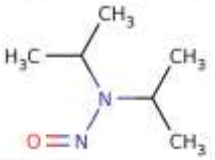
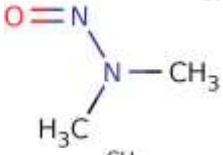
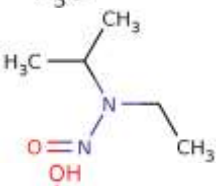
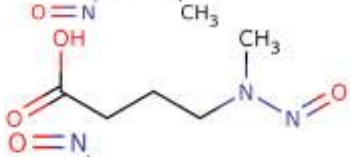
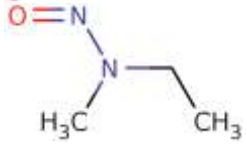
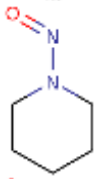
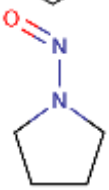
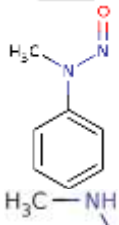
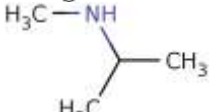
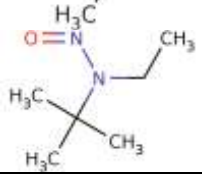
1. Introduction

Pharmaceutical impurities encompass undesired chemicals that can arise during the synthesis or production of pharmaceuticals, as well as under varying storage conditions such as temperature and humidity or as a result of interactions with excipients or contaminations (Kao et al., 2022). Among these impurities, nitrosamines (NAs) have garnered significant attention. NAs, characterized by the chemical structure "$R_2N–N = O$" is often formed by the interaction of a nitrosating agent (Sung et al., 2010; Bharate, 2021). Notably, NAs are potent genotoxic agents that can impact various living organisms and have been classified as potential human carcinogens by the International Agency for Research on Cancer (IARC) (Lapo, 2021). The emergence of NAs as a public health concern can be traced back to detecting impurities in angiotensin II receptor blocker drugs containing valsartan. In July 2018, the U.S. Food and Drug Administration (USFDA) reported the presence of N-nitroso dimethylamine (NDMA), one of the NA species, in valsartan-containing drugs (Lapo, 2021). Subsequently, NDMA was also identified in another angiotensin II receptor blockers like losartan and irbesartan, as well as in histamine II blockers such as ranitidine and nizatidine, and the anti-hyperglycemic drug metformin. Extensive studies have confirmed the mutagenic carcinogenicity of NDMA in various animal species, leading to its classification as "reasonably anticipated to be human carcinogens" by the IARC, World Health Organization (WHO), and the National Toxicology Program, U.S. Department of Health and Human Services (Tuesuwan and Vongsutilers, 2021). NA impurities can arise in drugs containing tetrazole, secondary, and tertiary amine structures (Figure 1).

Several NAs, including NDMA, N-nitroso-N-methyl-4-aminobutyric acid (NMBA), N-nitroso diethylamine (NDEA), N-nitroso diisopropylamine (NDIPA), N-nitroso ethyl isopropylamine (NEIPA), N-nitroso dibutylamine (NDBA), N-nitroso ethyl methylamine (NMEA), N-nitroso pyrrolidine (NPyR), and N-nitroso piperidine (NPIP) (Table 1), may be encountered as impurities in drug groups with this molecular structure (Li et al., 2021; Hu et al., 2021). Assessment reports released by the USFDA and European Medicines Agency (EMA) enounce that NDMA, NDEA, NMBA, NDBA, N-methyl-N-nitrosoaniline (NMPhA) are the most commonly detected impurities in specific drugs (Chidella et al., 2021; USFDA, 2021; EMA, 2021). Consequently, detecting NAs has become a crucial issue in drugs prone to impurities due to their susceptible production processes and materials.

Table 1. Twelve nitrosamine impurities reportedly identified in pharmaceuticals

Impurity	Structure	SMILES	MW (g/mol)	Log P	AI (ng/day)
NDBA		CCCCN(CCCC)N=O	158.25	2.57	26.5
NDEA		CCN(CC)N=O	102.14	1.79	26.5
NDIPA		CC(C)N(C(C)C)N=O	130.19	1.79	26.5
NDMA		CN(C)N=O	74.08	0.23	96.0
NEIPA		CCN(C(C)C)N=O	116.16	1.40	26.5
NMBA		CN(CCCC(=O)O)N=O	146.15	0.46	96.0
NMEA		CCN(C)N=O	88.11	0.62	-
NPIP		C1CCN(CC1)N=O	114.15	1.15	-
NpyR		C1CCN(C1)N=O	100.12	0.76	-
NMPhA		CN(C1=CC=CC=C1)N=O	136.15	1.80	-
NMIPA		CC(C)NC	73.14	0.61	-
N-tert-Butyl-N-ethylnitrosamine		CCN(C(C)(C)C)N=O	130.19	1.79	-

NDBA= N-nitrosodibutylamine, NDEA= N-Nitrosodiethylamine, NDIPA= N-nitrosodiisopropylamine, NDMA= N-Nitrosodimethylamine, NEIPA= N-nitrosoethylisopropylamine, NMBA= N-nitroso-N-methyl-4-aminobutyric acid, NMEA= N-nitrosoethylmethylamine, NPIP= N-nitrosopiperidine, NPyR= N-nitrosopyrrolidine, NMPhA= N-methyl-N-nitrosoaniline, NMIPA= N-Nitroso-N-methyl-2-propanamine, and N-tert-Butyl-N- ethylnitrosamine, MW= molecular weight, Log P= partition coefficient, AI= acceptable intake limit.

Guidance on controlling those impurities in medicinal and pharmaceutical products has been established by regulatory authorities like the USFDA and EMA due to the presence of different NA impurities (Chidella et al., 2021). These guidelines provide insights into the conditions that can contribute to the occurrence of NA impurities in pharmaceutical products. NA impurities can arise during active pharmaceutical ingredients (APIs) production through specific raw materials, starting materials, and intermediates. For instance, APIs may contain NAs due to using contaminated raw materials. Furthermore, the materials used in the production process, including solvents, reagents, and catalysts, can also play a role in NA formation. Amide solvents, for instance, are known to degrade into secondary amines, which are recognized as sources of NAs. Using sodium nitrite ($NaNO_2$) or other nitrites in the presence of secondary or tertiary amines presents a potential mechanism for NA formation (Schlingemann et al., 2023). Hu et al. (2021) reported that the formation of NAs can be attributed to side reactions during drug synthesis, breakdown of unstable drug compounds, contamination during production, and storage and packaging conditions. NA impurity levels in drugs can increase during production, especially under low pH and high-temperature conditions (Hu et al., 2021). According to a 2020 report by the USFDA, degradation occurring during the manufacturing of the finished metformin products led to high levels of NDMA (Tuesuwan et al., 2021; USFDA, 2021). Given the potential occurrence of NAs during production, it is imperative for pharmaceutical companies to take corrective actions to minimize the risk of NA formation in their products. Consequently, pharmaceutical companies have expanded their quality control analysis of NAs, encompassing assessments of raw materials, finished products, and residuals from the production process. Pharmaceutical products are released into the market following risk assessments, which include the analysis of NA impurities as part of the manufacturing process.

However, current quality control practices are primarily based on the analysis data of drugs before they are released to the market. Nonetheless, it is paramount to assess the impurity levels that may arise under different conditions during the shelf life of drugs, such as varying temperature and humidity conditions, after they have been launched to the market. Limited research exists on the potential risk of impurities posed by impurities that may develop due to exposure to high temperatures during the shelf life of drugs. While the NA impurities in the sartan group of drugs represent the tip of the iceberg, it is known that NA impurity formation is possible in other drug groups, mainly those containing tetrazole, secondary, and tertiary amine groups (Figure 1). Therefore, our study aims to determine the levels of NA impurities that may form under different temperature conditions during the shelf life of drug formulations using chromatographic methods. For this purpose, 11 drugs (valsartan, losartan, pioglitazone, escitalopram, rifampicin, fluoxetine, imipramine HCl, acyclovir, famotidine, metformin HCl, and venlafaxine) available in pharmacies, specifically those containing tetrazole, secondary, and tertiary amine groups (Figure 1), were subjected to prolonged exposure at two different temperatures (room temperature and 50°C). Subsequently, potential NA impurities were analyzed for all samples using an LC-MS/MS instrument.

2. Materials and Methods

2.1. Chemicals and Pharmaceuticals

The reference standard solutions of the 12 NAs (NDBA, NDEA, NDIPA, NDMA, NEIPA, NMBA, NMEA, NPIP, NpyR, NMPhA, NMIPA and N-tert-Butyl-N- ethyl nitrosamine in MeOH) in a concentration of 1000 µg/mL, and NDMA-D6 (isotope-labeled NDMA standard; as internal standard (IS)) at a concentration of 1 μgmL^{-1}, were purchased from Toronto Research Chemicals (Toronto, Canada). Methanol and acetonitrile of LC-MS grade and formic acid (> 98% purity) were purchased from Merck (Darmstadt, Germany). All drugs to be analyzed, namely valsartan, losartan, pioglitazone, escitalopram, rifampicin, fluoxetine, imipramine HCl, acyclovir, famotidine, metformin HCl, and venlafaxine were purchased from local pharmacies (Table 2).

2.2. Preparation of Calibration Standard Solutions and Quality Controls

The method employed for analyzing NA impurities in pharmaceuticals is directly utilized from the study by Mavis et al. (2023) (Mavis et al., 2023). Reference standard solutions at 1000 µg/mL concentration were diluted with methanol to prepare a working mix solution (WS1) at a concentration of 1 μgmL^{-1}. WS1 was diluted with deionized water to obtain six working mix solutions (WS2) at different concentrations (0.25, 2.5, 10, 25, 50, and 250 $ngmL^{-1}$). All solutions were stored at -20 °C. To calibration standards, each WS2 solution was diluted in a ratio of 1/5. One hundred microliters of each WS2 solution were taken, and 50 µL IS (1 μgmL^{-1}) and 350 µL deionized water were added to it. The concentrations of the obtained calibration standards were 0.05, 0.5, 2, 5, 10, 50 $ngmL^{-1}$. Furthermore, quality control samples (QCs) were prepared by adding calculated volumes of the WS2 to blank powders of the corresponding drug tablets at concentrations of 4 and 20 $ngmL^{-1}$.

2.3. Chromatographic Condition

Samples were analyzed by an Agilent Ultivo triple quadrupole LC/MS (6465B, Agilent Technologies, Santa Clara, CA, USA) equipped with an APCI source (Agilent Technologies, Santa Clara, CA, USA). Positive chemical ionization in dMRM mode was selected for MS/MS detection of the NAs. The analytical column (Poroshell HPH C18, 4.6 Å~ 150 mm 2.7 µm (P/N 693975–702, Agilent Technologies, Santa Clara, CA, USA)) was maintained at temperature of 20 °C, while the autosampler temperature was set at 8 °C. Mobile phase A (pH 3) consisted of 0.2% formic acid in deionized water,

and phase B consisted of methanol, with a flow rate of 0.6 mL/min. The initial gradient of 18% B was held for 1.0 minutes, followed by a linear increase to 78% B over 6.0 min. Subsequently, the gradient was converted to 100% B and maintained for 5.0 min. Finally, the gradient was returned to the initial condition over 5.0 minutes. The total running time was 17.0 min., and the sample injection volume was 20 µL. The mass spectrometer settings were as follows the drying gas temperature was set to 300 °C, the drying gas flow rate was 6 L/min, the nebulizer pressure was 55 psi, the vaporizer temperature was 350 °C, the corona current was 4 µA, and the capillary voltage was 3000 V. The quantification of the analytes was conducted by constructing calibration curves based on the concentrations of the calibrators, considering the matrix effect by adjusting for the yields of the corresponding IS (NDMA-D6).

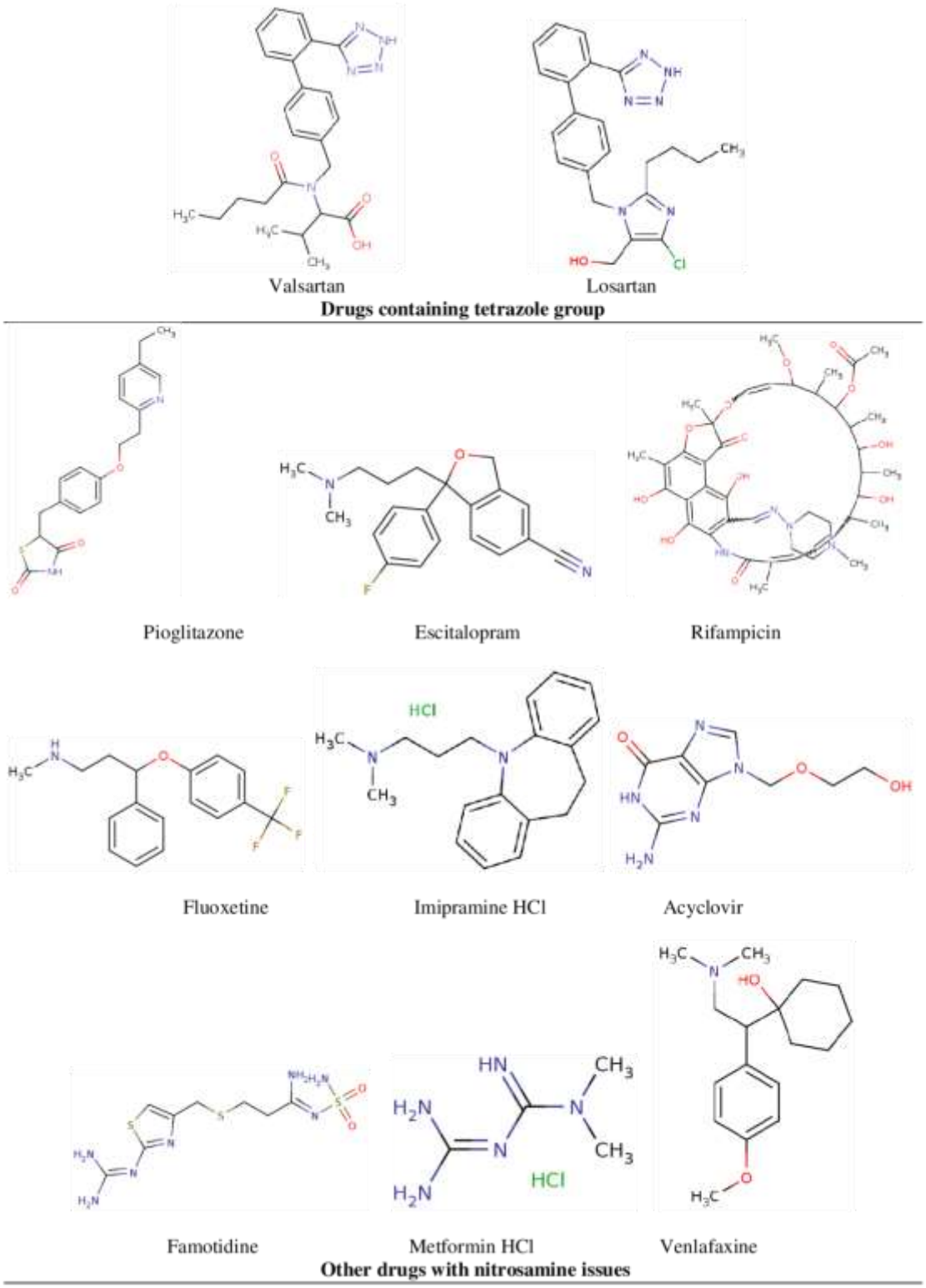


Figure 1. Chemical structures of the eleven pharmaceuticals with potential NA impurities in our study.

Table 2. Drugs and packaging shapes used in the study

Drug	DS (mg)	Formulation
Valsartan/Amlodipine	160 /5	Film tablet
Losartan potassium	50	Film tablet
Pioglitazone	30	Tablet
Essitalopram	10	Film tablet
Rifampicin	300	Capsule
Fluoxetin	20	Capsule
Imipramine HCl	25	Film tablet
Acyclovir	800	Tablet
Famotidine	40	Film tablet
Metformin HCl	500	XR* tablet
Venlafaxine	37.5	Micropellet

DS= dose strength, * extended-release.

2.4. Sample Treatment Procedure

A mandatory degradation study was conducted using stability chambers to investigate the impact of temperature on the formation of NA impurities in the drug packaging of the listed drugs in Table 2. The study involved subjecting the drugs to high temperatures without opening the packaging. The temperature ranges for drug stability, including drugs like ranitidine and similar drug groups, were reported to be 40-50°C (Hao et al., 2023). Workgroups were determined considering the shelf life and the upper limit temperatures for drug stability. The drugs were kept in their original packaging within stability chambers at different temperatures and time intervals, resulting in four groups.

As the drug packaging remained unopened, the drugs were not exposed to any oxygen entry. The first group was kept at 25°C/75% relative humidity (RH) for one week, adhering to the temperature conditions specified for shelf life. The second group was kept at 25°C/75% RH for four weeks following the shelf-life temperature conditions. The third group was subjected to the upper limit temperature for drug stability, which was 50°C/75% RH, for one week. Similarly, the fourth group was exposed to the upper limit temperature of 50°C/75% RH but four weeks.

To extract the analytes, tablet powder equivalent to 50 mg of all drugs was weighed and placed in a glass tube. Subsequently, 250 µL of IS was added to the tube and vortexed for 5 seconds. Next, 2250 µL of an extraction solution composed of deionized water and acetonitrile (80:20, v/v) with a pH of 4.0 adjusted using formic acid was added to the tube. The mixture was agitated at room temperature for 30 minutes. Following the extraction step, the suspension was centrifuged at 3600 Å~ g for 5 minutes and filtered through a 0.45 µm regenerated cellulose membrane prior to injection.

3. Results

Considering the potential carcinogenic effects of NAs, both the USFDA and EMA have issued guidelines outlining chromatographic analysis methods for the detection of these impurities in drug products. Additionally, various validated chromatographic analysis methods have been reported in the literature. In our study, we directly utilized the LC-APCI-MS/MS method developed by Mavis et al. in their 2023 publication, which was originally designed for the detection of multiple NA impurities in valsartan and irbesartan drugs (Mavis et al., 2023). We applied this method to analyze the impurities present in our drug samples. In our study, a comprehensive analysis of 12 NAs in drug samples was conducted using LC-APCI-MS/MS. A dynamic multiple reaction monitoring (mrm) method with a runtime of 17 minutes was performed, utilizing a Poroshell HPH C18 (4.6 × 150 mm, 2.7 µm) column in a gradient system mobile phase flow (Mobile phase A: 0.2% formic acid, Mobile phase B: Methanol) to detect and quantify the 12 NAs (Figure 2).

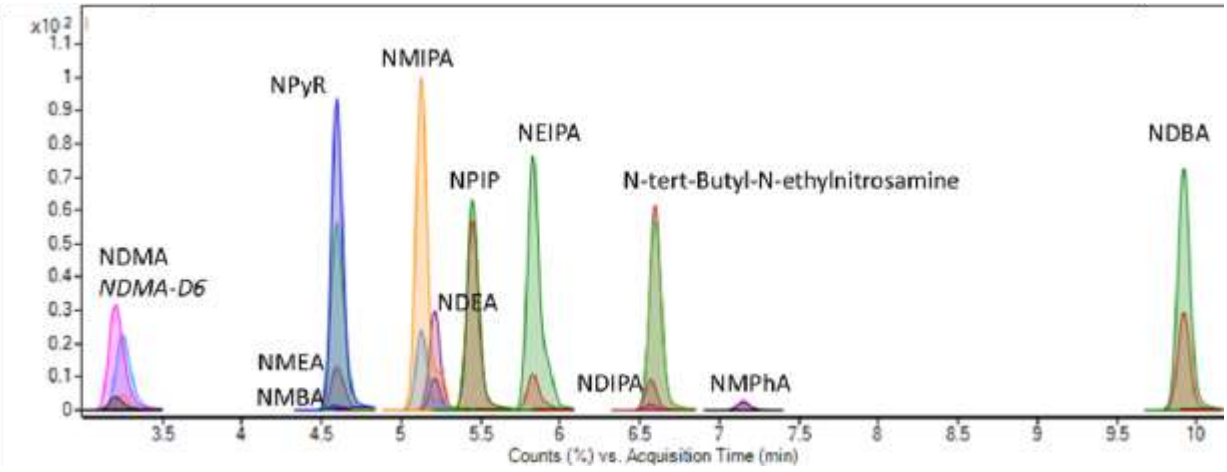


Figure 2. Chromatogram of the 12 NA impurities included in the study. NDBA= N-nitrosodibutylamine, NDEA= N-Nitrosodiethylamine, NDIPA= N-nitrosodiisopropylamine, NDMA= N-Nitrosodimethylamine, NEIPA= N-nitrosoethylisopropylamine, NMBA= N-nitroso-N-methyl-4-aminobutyric acid, NMEA= N-nitrosoethylmethylamine, NPIP= N-nitrosopiperidine, NPyR= N-nitrosopyrrolidine, NMPhA= N-methyl-N-nitrosoaniline, NMIPA= N-Nitroso-N-methyl-2-propanamine, and N-tert-Butyl-N- ethylnitrosamine, NDMA-D6= isotope-labeled NDMA standard (as internal standard).

The determined level in our study had a calibration curve range of 0.05–50 ngmL^{-1} for all NAs. The analysis's limit of quantitation (LOQ) was determined to be 0.05 ngmL^{-1} for all 12 NAs. The results presented in Table 3 indicate that the calibration curves exhibited strong linearity, with a coefficient of determination (R^2) exceeding 0.997 for all NAs (Figures 3-5).

Table 3. Calibration results for 12 NA impurities

Impurity	Precursor/product ion (m/z)	RT (min)	R^2 Value	Calibration Range (ngmL^{-1})	LOQ (ngmL^{-1})
NDBA	159.0/57.0	9.93	0.998	0.05-50	0.05
NDEA	103.1/75.5	5.22	0.999		
NDIPA	131.1/43.1	6.62	0.998		
NDMA	75.0/43.0	3.28	0.998		
NEIPA	117.0/74.9	5.84	0.999		
NMBA	147.0/44.1	4.60	0.999		
NMEA	89.1/61.0	4.61	0.998		
NPIP	115.1/41.0	5.46	0.999		
NpyR	101.1/55.0	4.61	0.998		
NMPhA	137.0/107.0	7.16	0.999		
NMIPA	103.1/60.9	5.14	0.999		
N-tert-Butyl-N-ethylnitrosamine	131.0/75.0	6.59	0.997		

RT= retention time, R^2= coefficient of determination (R squared); LOQ= limit of quantification

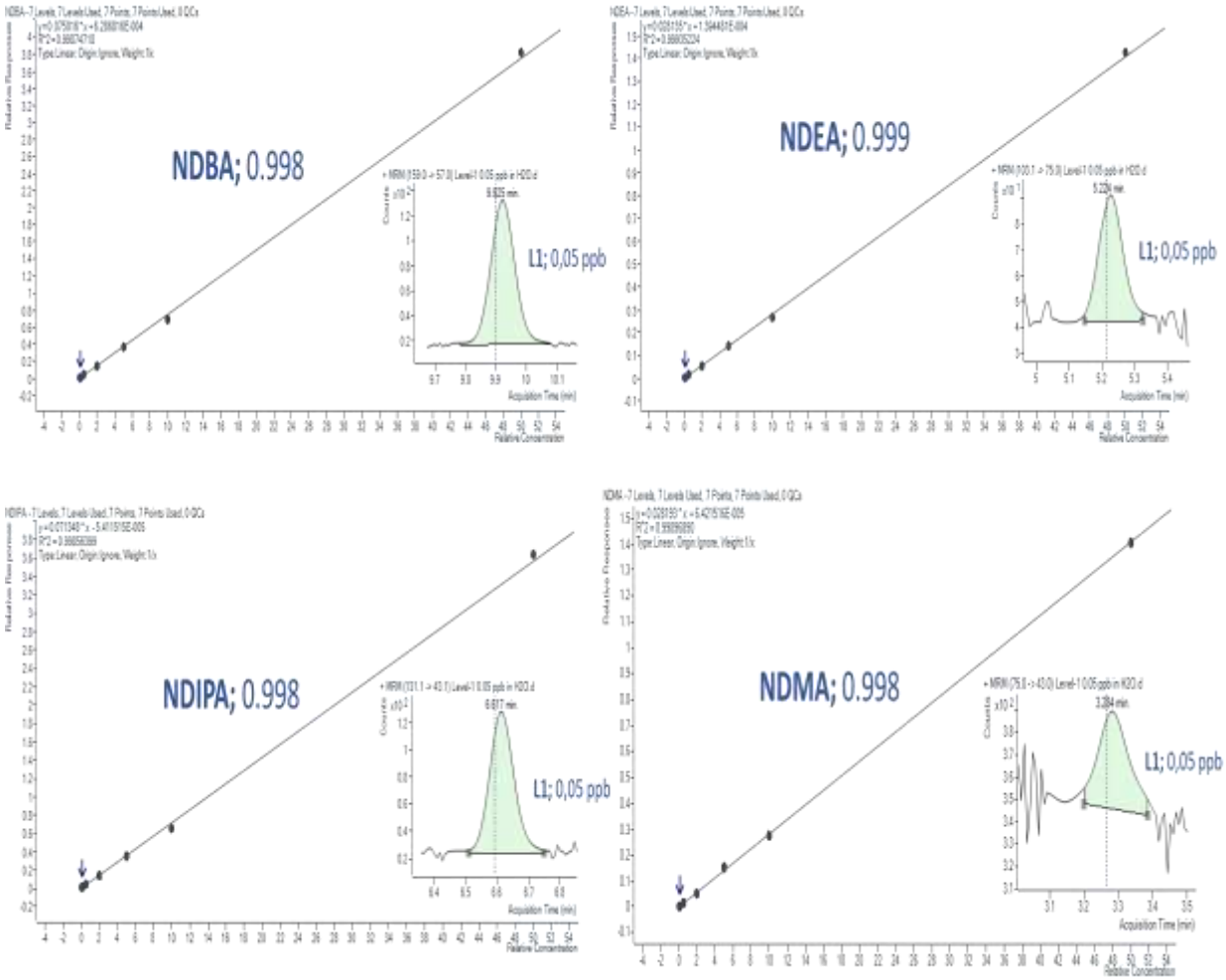


Figure 3. Calibration curves of the NDBA, NDEA, NDIPA and NDMA impurities included in the study. NDBA= N-nitrosodibutylamine (R2= 0.998, retention time (RT, min)= 9.93= precursor/product ion (m/z)= 159.0/57.0), NDEA= N-Nitrosodiethylamine (R2= 0.999, retention time (RT, min)= 5.22= precursor/product ion (m/z)= 103.1/75.5), NDIPA= N-nitrosodiisopropylamine (R2= 0.998, retention time (RT, min)= 6.62= precursor/product ion (m/z)= 131.1/43.1), NDMA= N-Nitrosodimethylamine (R2= 0.998, retention time (RT, min)= 3.28= precursor/product ion (m/z)= 75.0/43.0).

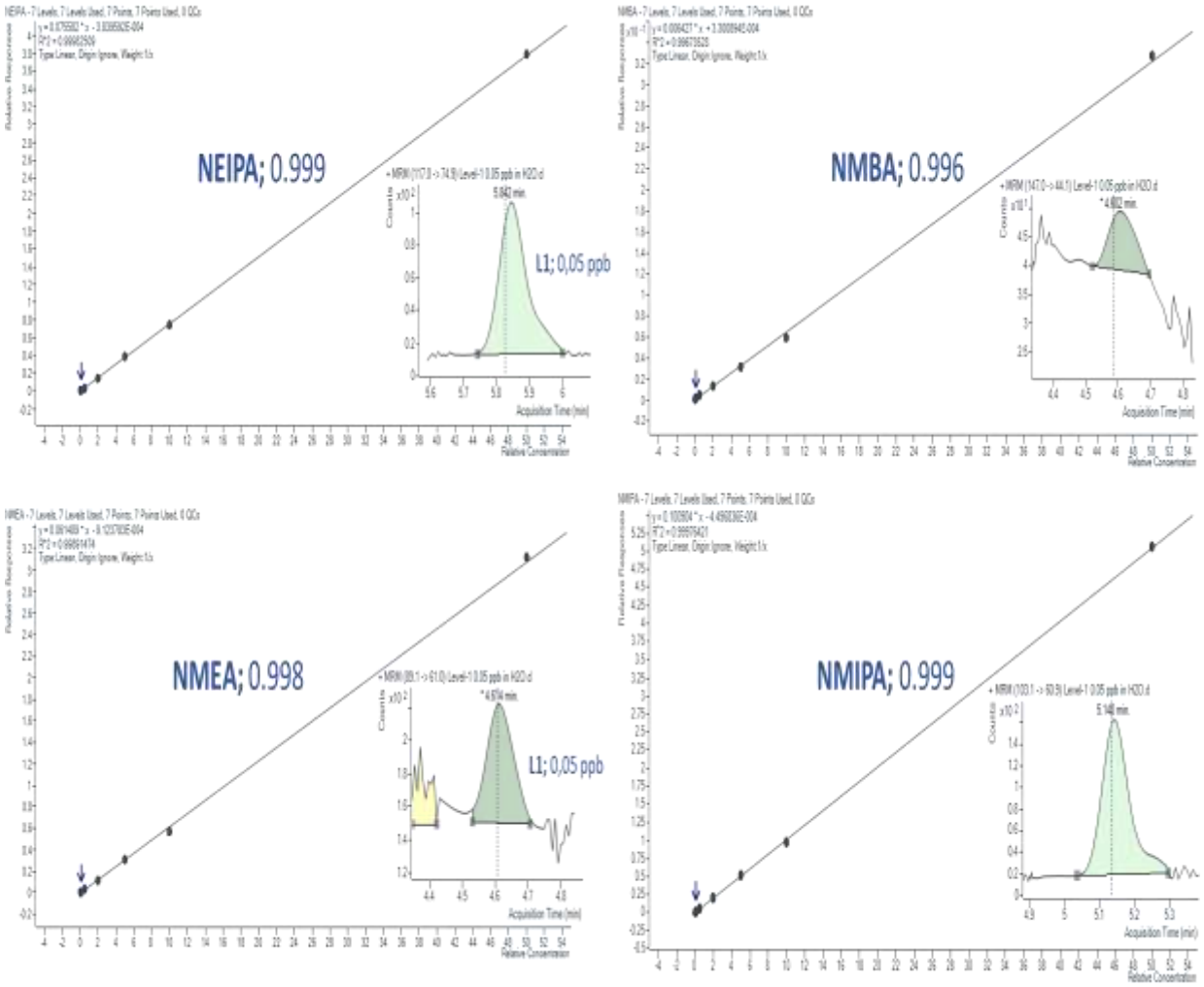


Figure 4. Calibration curves of the NEIPA, NMBA, NMEA and NMIPA impurities included in the study. NEIPA= N-nitrosoethylisopropylamine (R2= 0.999, retention time (RT, min)= 5.84= precursor/product ion (m/z)= 117.0/74.9), NMBA= N-nitroso-N-methyl-4-aminobutyric acid (R2= 0.999, retention time (RT, min)= 4.60= precursor/product ion (m/z)= 147.0/44.1), NMEA= N-nitrosoethylmethylamine (R2= 0.998, retention time (RT, min)= 4.61= precursor/product ion (m/z)= 89.1/61.0), NMIPA= N-Nitroso-N-methyl-2-propanamine (R2= 0.999, retention time (RT, min)= 5.14= precursor/product ion (m/z)= 103.1/60.9).

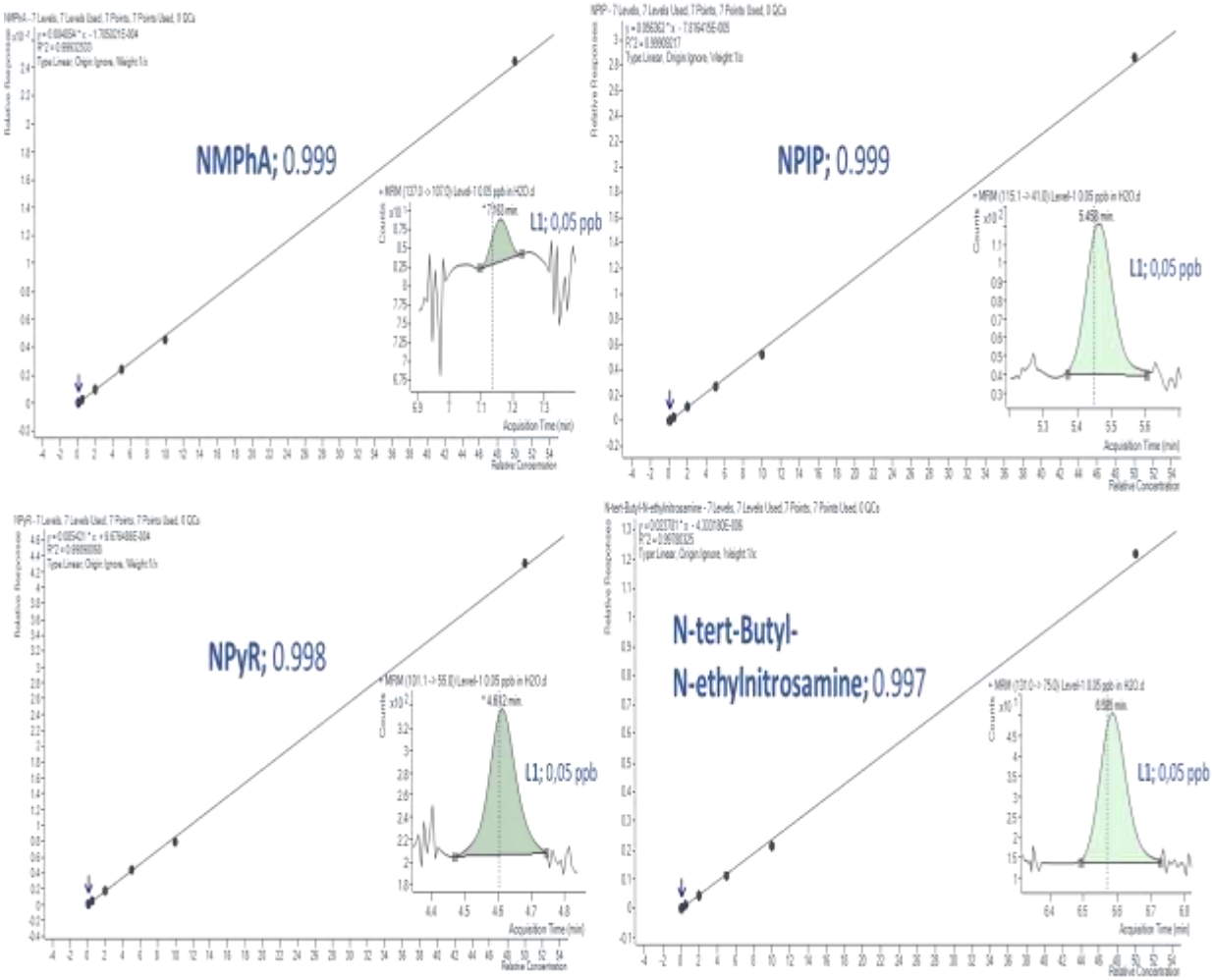


Figure 5. Calibration curves of the NMPhA, NPIP, NPyR and N-tert-Butyl-N- ethylnitrosamine impurities included in the study. NMPhA= N-methyl-N-nitrosoaniline (R2= 0.999, retention time (RT, min)= 7.16= precursor/product ion (m/z)= 137.0/107.0), NPIP= N-nitrosopiperidine (R2= 0.999, retention time (RT, min)= 5.46= precursor/product ion (m/z)= 115.1/41.0), NPyR= N-nitrosopyrrolidine (R2= 0.998, retention time (RT, min)= 4.61= precursor/product ion (m/z)= 101.1/55.0), and N-tert-Butyl-N- ethylnitrosamine (R2= 0.997, retention time (RT, min)= 6.59= precursor/product ion (m/z)= 131.0/75.0).

Our study presents the NA analysis conducted on 11 commercially available drugs (valsartan, losartan, pioglitazone, escitalopram, rifampicin, fluoxetine, imipramine HCl, acyclovir, famotidine, metformin HCl, and venlafaxine) listed in Table 2 in the materials and methods section. These drugs were kept unopened and subjected to various time intervals and temperature conditions, reflecting real-world scenarios. The analysis results of NA impurities in 11 drugs, stored at various time intervals and temperature conditions, are presented in Table 4. When the 11 drugs were analyzed for NA impurities after being stored for one week and four weeks under specific storage conditions, no impurities were detected within the calibration range of 0.05-50 $ngmL^{-1}$. Furthermore, when the NA impurity results of the 11 drugs stored for one week and four weeks at 50°C and 75% humidity stability chambers were examined (as shown in Table 4), it was observed that no impurities developed at this temperature and storage duration.

Table 4. NA impurity results of 11 drug samples stored at different temperatures

Impurity	Valsartan/ Amlodipine	Losartan	Pioglitazone	Escitalopram	Rifampicin	Fluoxetine	Imipramine HCl	Acyclovir	Famotidine	Metformin HCl	Venlafaxine
Room temperature; 25 ℃ / 75% humidity (one week and four weeks)											
NDBA	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
NDEA	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
NDIPA	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
NDMA	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
NEIPA	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
NMBA	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
NMEA	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
NPIP	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
NpyR	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
NMPhA	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
NMIPA	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
N-tert-Butyl-N-ethylnitrosamine	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
50 ℃/ 75% humidity (one week and four weeks)											
NDBA	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
NDEA	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
NDIPA	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
NDMA	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
NEIPA	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
NMBA	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
NMEA	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
NPIP	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
NpyR	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
NMPhA	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
NMIPA	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
N-tert-Butyl-N-ethylnitrosamine	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d

NDBA= N-nitrosodibutylamine, NDEA= N-Nitrosodiethylamine, NDIPA= N-nitrosodiisopropylamine, NDMA= N-Nitrosodimethylamine, NEIPA= N-nitrosoethylisopropylamine, NMBA= N-nitroso-N-methyl-4-aminobutyric acid, NMEA= N-nitrosoethylmethylamine, NPIP= N-nitrosopiperidine, NPyR= N-nitrosopyrrolidine, NMPhA= N-methyl-N-nitrosoaniline, NMIPA= N-Nitroso-N-methyl-2-propanamine, and N-tert-Butyl-N- ethylnitrosamine, n.d= not dedected.

4. Discussion

Numerous studies have focused on detecting and investigating N-nitrosamine precursors, particularly NDMA, in drugs. The IRAC has classified NDMA as a possible human carcinogen (group 2A) (Yoon et al., 2021). Following identifying NDMA in ranitidine-containing medications in September 2019, the USFDA and EMA established an acceptable daily intake limit of 96 ng or 0.32 ppm for ranitidine. However, manufacturers voluntarily recalled ranitidine worldwide when NDMA levels were found to exceed the USFDA's recommended limit by a factor of nine in numerous ranitidine-containing medicines (Yoon et al., 2021). The USFDA has temporarily set a tolerable limit of 96 ng/day in a tablet or capsule (Monajjemzadeh and Robertson, 2022). The EMA has set temporary tolerable limits of 26.5 ng/day or 96 ng/day for selected NA impurities, depending on their similarity to NDEA or NDMA (Thresher et al., 2020).

In response to the occurrence of NA contamination in sartans, regulatory bodies, and research organizations have made substantial endeavors to create analytical approaches utilizing different chromatographic analysis methods like GC-MS/MS, LC-MS/MS, and HPLC. These methods aim to accurately measure the levels of NAs in both the active APIs and pharmaceutical products (Tuesuwan and Vongsutilers, 2021). However, analyzing ppm-level contaminants like NAs in pharmaceuticals can lead to accurate results with rigorous quantitative analytical methods.

In a study by Schmidtsdorff and Schmidt, a supercritical fluid chromatography (SFC) method was developed to simultaneously determine NAs (NDMA, NDEA, NMEA, N-nitrosodi-n-propylamine (NDnPA), NDPA, NDBA, N-nitroso diphenylamine (NDPhA), NPyr, N-nitroso piperidine (NPip), NMor) at ppb levels in the sartan drugs valsartan and losartan (Schmidtsdorff and Schmidt, 2019). In another study by Xu et al. (2021) an LC-MS/MS method was developed to analyze NAs (N-nitroso dimethylamine (NDMA), N-nitroso diethylamine (NDEA), N-nitroso diisopropylamine (NDIPA), N-nitroso ethyl isopropylamine (NEIPA), N-nitroso dibutylamine

(NDBA), and N-nitroso methyl aminobutyric acid (NMBA)) as impurities in a valsartan sample. The calibration range for these NA impurities in valsartan was 0.05 – 3.6 ppm (Xu et al., 2021). Furthermore, Wohlfart et al. conducted a study in 2021 where they developed an LC-MS/MS method to quantify the amount of 4-methyl-1-nitrosopiperazine (MeNP) impurity in rifampicin. The calibration range for MeNP in rifampicin was established to be 0.7 to 5.1 ppm (Wohlfart et al., 2021). In a recent study by Wogel and Norvig, they developed an LC-MS/MS method utilizing solid phase extraction to detect 13 NAs (NDMA, NMEA, NPyr, NDEA, nitroso morpholine (NMor), ethyl-nitroso-(propane-2-yl)-amine (EIPNA), N-Nitrosodiisopropylamine (DIPNA), N-nitroso-di-n-propylamine (NDPA), N-Nitroso-N-methylaniline (NMAni), N-N-Nitrosomethyl-4-aminobutyric acid (NMBA), NDBA, N-nitroso diphenylamine (NDPhA)) that could potentially be present as impurities in pharmaceuticals. The method exhibited a remarkable detection limit of up to 0.025 ppb, demonstrating its high sensitivity and reliability in identifying trace levels of NAs (Vogel and Norwig, 2022). Similarly, Mavis et al. developed an LC-APCI-MS/MS method for the quantitative determination of 11 NAs (NMEA, NMIPA, NPIP, NPyR, NDMA NDEA, NMBA, DIPNA, NDBA, NMPhA, and EIPNA) in valsartan and irbesartan drugs. The method utilized had a calibration range of 0.5-50 $ngmL^{-1}$, allowing for precise quantification of the NA impurities (Mavis et al., 2023). These studies demonstrate the development of effective chromatographic analysis methods to accurately measure and quantify NAs in pharmaceuticals, addressing the need for reliable analytical techniques in detecting and monitoring these contaminants. Our study directly utilized the method of Mavis et al. to analyze NA impurities at ppb levels (Figure 3-5). It is essential to have a sensitive analytical method in impurity studies for accurate detection. Therefore, reporting impurity detection limits in ppm or ppb levels is customary in the literature. In our study, we detected and quantified NAs at low concentrations with a sensitive analysis method similar to the literature. (Table 3, Figure 2).

In our study, we utilized an LC-MS/MS method to investigate the presence of NA impurities in drugs and assess the impact of temperature on impurity formation during storage conditions. The literature contains several recent studies that are relevant to drug impurity analysis under storage conditions. A notable study by Abe et al. (2020) sheds light on the effect of high-temperature storage on ranitidine stability, particularly on NDMA formation. Samples were analyzed using LC-MS/MS. Following the drug stability guidelines outlined by the International Conference on Harmonization (ICH-Q1A), it was observed that NDMA levels significantly increased over 8 weeks from 0.19 to 116 ppm and from 2.89 to 18 ppm for tablet A and tablet B, respectively, when stored under accelerated conditions (40 °C with 75% RH) (Abe et al., 2020). The study demonstrated a correlation between temperature and NDMA formation, with higher temperatures increasing impurity levels. Another study conducted by Golob et al. (2023) investigated film-coated tablets containing crospovidone from two different manufacturers with varying levels of nitrite. The tablets were subjected to room temperature and accelerated stability temperature (40 °C/75% RH). NDMA and nitrite were detected at ppb levels, while DMA was detected at ppm levels in the drug product. The NDMA formation in the drug product was time, temperature, and nitrite-dependent, with a reduction in DMA and nitrite emphasized. The study highlighted that impurity formation increased over time and with high-temperature exposure in the presence of nitrite at room temperature. However, when there was no impurity formation at room temperature, impurity formation was not observed at the stability temperature (Golob et al., 2023).

In a study conducted by Hao et al. (2023) the impact of mandatory degradation tests on NA formation was investigated using a long-acting metformin drug (Hao et al., 2023). The analysis conditions included oxidation conditions at different temperatures and low pH conditions. Impurities were not detected in sample groups stored at different temperatures when stored under specific conditions (25°C/60% RH, 4 days) in an acidic environment. Even when these samples were subject to high temperatures (50°C/60% RH, 4 days), no impurities were observed. However, impurity formation was observed in samples stored in a peroxide environment under storage conditions, with high temperatures leading to increased impurity levels, consistent with observations in other conditions. Our study analyzed the drug samples at different time points and temperatures to assess the presence of NA impurities. Our findings indicate that none of the drugs analyzed (one week or four weeks) exhibited any detectable NA impurities under storage conditions of temperature and 75% humidity (Table 4). Additionally, when these drugs were subjected to high-temperature conditions of 50 °C and 75% humidity for either one week or four weeks, no NA impurities were formed (Table 4). The initial formation of NA impurities in pharmaceuticals mostly occurs through the reaction of nitrous acid with organic solvents during the API synthesis. Therefore, if impurities form during the API synthesis, these impurities can be detected using chromatographic methods under storage conditions. In our study, since no nitrosamine impurity was detected in the analyzed drugs, it can be concluded that the quality controls during the API synthesis of the drugs included in the analysis were conducted with precision.

Based on our study results, it can be concluded that for the selected drugs, if no NA impurities are present during the drug production steps, the temperature variations encountered during storage conditions do not contribute to impurity formation. Our findings suggest that the drugs analyzed in our study were not contaminated

during production, and NA formation did not occur. It is worth noting that our study data aligns with existing literature findings. However, while previous research has focused on a limited number of drugs and NA groups in terms of impurity analysis under storage conditions, our study expands upon this by analyzing a more extensive range of drugs and NA groups. The studies mentioned above offer valuable insights into the interplay between storage conditions, temperature, and the formation of NA impurities in pharmaceuticals. They underscore the significance of closely monitoring and comprehending the factors contributing to generating these impurities. Considering the findings from both the literature and our study, it becomes evident that controlling and monitoring stability-related factors and controlling impurities during production is crucial. This holistic approach is essential for mitigating some pharmaceuticals' health risks associated with NA impurities.

5. Conclusion

Nitrosamines are an essential source of impurities for public health. Pharmaceutical companies launch drugs to the market by performing various quality control analyses to prevent the formation of NA impurities. However, evaluating the level of NA impurity formation during the shelf life of drugs released to the market after passing quality control and risk assessment is vital to protect public health. Therefore, in our study, we specifically investigated the effect of the temperature parameter, which affects NA impurity formation, on pharmaceuticals available on the market. Our study revealed that for pharmaceuticals on the market that did not have nitrosamine impurities detected during production and passed quality control, nitrosamine impurities did not form even when storage conditions were changed (temperature was increased) for four weeks. This demonstrates the importance of simultaneous impurity quality audits during the manufacturing phase of pharmaceuticals and that the level of impurity formation during shelf life may be low for medicines that have passed quality audits. It is vital to continue to monitor and control NA impurities in pharmaceuticals to minimize health risks. As long as the Pharmaceutical Industry raises safety standards by prioritizing quality control measures throughout the manufacturing process and considering factors such as temperature, it is anticipated that the integrity of pharmaceutical products will not be compromised. Continued research and extensive studies are required to further improve our understanding of NA impurity formation and effectively address the public health concerns associated with these impurities.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

%	F.C	A.A
C	100	
D	100	
DCP	80	20
DAI	60	40
L	70	30
W	60	40

C= concept, D= design, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing.

Conflict of Interest

The authors declared that there is no conflict of interest.

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Ethical Approval/Informed Consent

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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İDRAR YOLU ENFEKSİYONLARINDA AMPİRİK TEDAVİ TERCİHİ NE OLMALI?

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Özet: İdrar yolu enfeksiyonları hastane ve toplumda en sık karşılaşılan enfeksiyonların başında gelmektedir. Kültür imkanı olmayan durumlarda ampirik tedavi hastalığın sağaltım ve komplikasyonlarının önlenmesinde kritik öneme sahiptir. Bu çalışmada amaç kümülatif antibiyogram analizi ile klinisyene ampirik tedaviye başlamada yol gösterici olmaktır. 2014 Ocak - 2022 Aralık arasında laboratuvarımıza gelen idrar örneklerinin kültürleri yapıldı. Üreyen bakteriler konvansiyonel yöntemler, disk difüzyon, BD Phoenix (BD, ABD) ve Vitek2 (Biomerieux, Fransa) otomatize sistemleri ile identifiye edilerek antibiyotik duyarlılık testleri yapıldı. Bir hastada üretilen bakterilerin aynı türden olması durumunda ilk izolat verisi kullanıldı. Duyarlılık oranı %90 üzerinde olan antibiyotikler ampirik tedavide önerildi. Çalışmamızda idrar kültürlerinde üreyen etkenlerin %86,6'sının Gram (-) bakteriler ve toplamın %62,9'unun *Escherichia coli* olduğu görüldü. Tüm izolatlara bakıldığında ayaktan hastalarda siprofloksasin direncinin yüksek ve artmakta olduğu (%44,6); fosfomisin (%10,8) ve nitrofurantoin (%13,2) dirençlerinin düşük olduğu saptandı. Yatan hastalarda intravenöz olarak tercih edilen seftriakson direncinin (%63,3) yüksek olduğu, ertapenem direncinin (%29,3) daha düşük olduğu görüldü. Ayaktan ve yatan hastalarda üreyen *E. coli* izolatlarında nitrofurantoin ve ertapenem dirençlerinin %10 altında olduğu, *E. coli* dışındaki izolatlar için bu durumun geçerli olmadığı görüldü. *E. coli* izolatlarında tüm antibiyotiklere direnç oranının yatan hastalarda ayakta tedavi görenlere göre anlamlı yüksek olduğu saptandı (her biri için P<0,001). Non-fermenter bakterilerde yatan hastalarda siproflaksasin, imipenem ve meropenem direnci %60'ın üzerinde bulundu. Çalışmamızda elde edilen bulgular kültür imkanı olmayan durumlarda veya kültür antibiyogram sonuçları elde edilene kadar ayaktan tedavide nitrofurantoin ve fosfomisinin, yatan hastalarda ertapenem ve fosfomisinin diğer antibiyotiklere göre ampirik tedavide başarılı olabileceğini göstermektedir.

Anahtar kelimeler: İdrar yolu enfeksiyonu, Antibiyotik direnci, Ampirik tedavi

What Should Be the Preference of Empirical Treatment in Urinary Tract Infections?

Abstract: Urinary tract infections are one of the most common infections in hospitals and society. In cases where culture is not possible, empirical treatment is critical in the treatment of the disease and the prevention of its complications. The aim of this study is to guide the clinician in starting empirical treatment with cumulative antibiogram analysis. The cultures of the urine samples that came to our laboratory between January 2014 and December 2022 were performed. The growing bacteria were identified using conventional methods, disc diffusion, BD Phoenix (BD, USA) and Vitek2 (Biomerieux, France) automated systems, and antibiotic susceptibility tests were performed. Initial isolate data were used when bacteria grown in a patient were of the same species. Antibiotics with a sensitivity rate of over 90% were recommended for empirical treatment. In our study, 86.6% of the factors grown in urine cultures were Gram (-) bacteria and 62.9% of the total were *Escherichia coli*. Considering all isolates, ciprofloxacin resistance is high and increasing in outpatients (44.6%); It was determined that fosfomycin (10.8%) and nitrofurantoin (13.2%) resistances were low. In hospitalized patients, resistance to intravenous ceftriaxone (63.3%) was found to be high, while resistance to ertapenem (29.3%) was lower. It was observed that nitrofurantoin and ertapenem resistances were below 10% in *E. coli* isolates grown in outpatients and inpatients, and this was not valid for isolates other than *E. coli*. The resistance rate to all antibiotics in *E. coli* isolates was found to be significantly higher in inpatients than in outpatients (P<0.001 for each). Resistance to ciprofloxacin, imipenem and meropenem was found over 60% in patients hospitalized with non-fermenter bacteria. The findings obtained in our study show that nitrofurantoin and fosfomycin may be successful in outpatient treatment, and ertapenem and fosfomycin may be successful in empiric treatment compared to other antibiotics in inpatients, in cases where culture is not possible or until culture antibiogram results are obtained.

Keywords: Urinary tract infection, Antibiotic resistance, Empirical treatment

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1. Giriş

İdrar yolu enfeksiyonları (İYE) kadınlarda ve erkeklerde en sık görülen enfeksiyonlardandır. İYE olguları hastane ve toplum kaynaklı en sık karşılaşılan enfeksiyonların başında gelmektedir. Cinsel aktivite, vajinal enfeksiyonlar, prostat sıvısındaki antimikrobik aktivite, hijyen uygulamaları, diyabet, obezite ve genetik yatkınlık İYE gelişmesi için risk faktörleri arasındadır (Hooton, 2012; Bader ve ark., 2017). Ayrıca hastanede yatan hastalarda başta kateterizasyon olmak üzere birçok faktöre bağlı olarak hastane kaynaklı İYE gelişmesi riski

vardır (Hooton, 2012; Bader ve ark., 2017; Caron ve ark., 2018; Warzecha ve ark., 2021).

Altta yatan risk faktörlerinin yanı sıra enfeksiyonun toplumsal ya da hastane kaynaklı olması İYE olgularında hem etken profilinin hem de antibiyotik duyarlılık paternlerinin farklı olmasına yol açmaktadır. Bunun dışında İYE olgularında izole edilen bakterilerin antibiyotiklere direnç profilleri hızlı direnç gelişebilmesi nedeniyle değişim göstermektedir. Bu nedenlere bağlı olarak İYE olgularında ampirik tedavi seçeneklerinin güncel tutulması gerekmektedir (Caron ve ark., 2018; Warzecha ve ark., 2021; Abbott ve ark., 2022).

İYE olgularında izole edilen etkenlerin Gram negatif enterik ya da non-fermenter bakteriler olması ya da Gram pozitif bakterilerin izole edilmesi tedavi yönetimini büyük ölçüde değiştirmektedir. Bu farklı gruplardaki mikroorganizmaların direnç profillerinin genel olarak bilinmesi ampirik tedaviye yaklaşımı etkilemektedir (Sastry ve Doi, 2016; Chu ve Lowder, 2018; Abbott ve ark., 2022).

Kültür imkanı olmayan durumlarda ya da kültür sonuçları rapor edilene kadar uygulanan ampirik tedavi hastanın iyileşmesinde ve komplikasyonların önlenmesinde kritik öneme sahiptir (Sastry ve Doi, 2016; Chu ve Lowder, 2018; Warzecha ve ark., 2021; Abbott ve ark., 2022). Bu çalışmada İYE olgularında kümülatif antibiyogram analizi yapmak ve bu şekilde klinisyenlerin ampirik tedaviye başlamalarında yol gösterici olmak amaçlanmıştır.

2. Materyal ve Yöntem

2.1. Hastalar ve Testler

Çalışmaya 2014 Ocak - 2022 Aralık arasında laboratuvarımıza gelen idrar örnekleri dahil edildi. Çalışmada dokuz yıllık dönemde 16525 izolata ait sonuçlar hastane otomasyon sistemi kayıtlarından geriye dönük olarak tarandı.

Örnekler Kanlı ve Eozin Metilen Mavisi (EMB) agara ekildikten sonra 37 °C 24-48 saat inkübe edildi. Büyüme olan koloniler bakteriyel idendifikasyon ve antibiyotik duyarlılık testleri için konvansiyonel yöntemler (koloni karakteristikleri, Gram boyama, biyokimyasal testler ve disk diffüzyon testleri), 2014 Ocak -2017 Mart tarihleri arasında BD Phoenix (BD, ABD), 2017 Nisan - 2022 Aralık tarihleri arasında Vitek2 (Biomerieux, Fransa) otomatize sistemler ile çalışıldı ve EUCAST standartlarına göre değerlendirildi. Kümülatif antibiyogram verilerinin analizi için CLSI M39-A4 (Ocak 2014) önerileri kullanıldı. Bir hastada üretilen bakterilerin aynı türden olması durumunda ilk izolat verisi kullanıldı. Duyarlılık oranı %90 üzerinde olan antibiyotikler ampirik tedavide önerildi.

Onsekiz yaş altındaki hastalara ait örnekler çalışmaya dahil edilmedi.

2.2. İstatistik Analiz

Çalışmadaki tüm istatistiksel analizler SPSS 25.0 programı (IBM SPSS, Chicago, IL, USA) kullanılarak yapıldı. Tanımlayıcı veriler sayı ve yüzde olarak verildi. Kategorik değişkenler açısından gruplar arasındaki karşılaştırmalar Pearson's Ki Kare testi ve Fisher's Exact Test ile yapıldı. Sonuçlar %95 güven aralığında değerlendirildi ve $P<0.05$ değerleri anlamlı kabul edildi. Gerekli yerlerde Bonferroni düzeltmesi yapıldı (Önder, 2018).

3. Bulgular

Çalışmamızda tüm izolatların %77,5'i ayaktan hastadır. Ayaktan hastalarda üreyen Gram (-) bakteriler ayaktan tüm izolatların %86,5'ini oluşturmaktadır. İdrar kültürlerinde üreyen etkenlerin %86,6'sının Gram (-) bakteriler ve toplamın %62,9'unun *E. coli* olduğu görüldü (Tablo 1). Tablo 2'de Gram pozitif ve Gram negatif izolatların yıllara göre dağılımı gösterilmiştir.

Tablo 1. İdrar kültürleri sonuçlarının bakteriyel izolatlara ve hastalara göre dağılımı*

	Ayaktan		Yatan		Toplam	
	n	%	n	%	n	%
Tüm bakteriler	12814	77,5	3711	22,5	16525	100
Gram (-) bakteriler	11088	86,5	3218	86,7	14306	86,6
Gram (-) enterik bakteriler	10773	84,1	2523	68	13296	80,5
Escherichia coli	8991	70,2	1403	37,8	10394	62,9
Klebsiella spp.	1029	8	412	11,1	1441	8,7
Proteus spp.	389	3	472	12,7	861	5,2
Enterobacter spp.	242	1,9	135	3,6	377	2,3
Serratia spp.	89	0,7	65	1,8	154	0,9
Citrobacter spp.	25	0,2	24	0,6	49	0,3
Diğer	8	0,1	12	0,3	20	0,1
Gram (-) non-fermenter bakteriler	315	2,5	695	18,7	1010	6,1
Pseudomonas aeruginosa	201	1,6	310	8,4	511	3,1
Acinetobacter spp.	102	0,8	367	9,9	469	2,8
Stenotrophomonas spp.	12	0,1	18	0,5	30	0,2
Gram (+) bakteriler	1726	13,5	493	13,3	2219	13,4
Enterokoklar	1309	10,2	402	10,8	1711	10,4
Stafilokoklar	376	2,9	75	2	451	2,7
Streptokoklar	41	0,3	16	0,4	57	0,3

*Her sütunda yüzdeler o sütun toplamına göre verilmiş, ayaktan ve yatan toplam izolat oranları ise genel toplama göre verilmiştir.

Tüm izolatlara bakıldığında hem ayaktan hem yatan hastalardan elde edilen izolatlarda en yüksek direnç oranının ampisiline olduğu (%65,7), tüm hastalarda siprofloksasin direncinin yüksek ve artmakta olduğu (%44,6); fosfomisin (%10,8) ve nitrofurantoin (%13,2) dirençlerinin düşük olduğu saptandı. Yatan hastalarda intravenöz olarak tercih edilen seftriakson direncinin (%63,3) yüksek olduğu, ertapenem direncinin (%29,3) daha düşük olduğu görüldü. Tüm antibiyotiklere direnç oranlarının yatan hastalarda anlamlı yüksek olduğu saptandı (her biri için P<0,001) (Tablo 3).

Ayaktan ve yatan hastalarda üreyen *E. coli* izolatlarında nitrofurantoin, fosfomisin ve ertapenem dirençlerinin %10 altında olduğu görüldü. *E. coli* izolatlarında tüm antibiyotiklere direnç oranının yatan hastalarda ayakta tedavi görenlere göre anlamlı yüksek olduğu saptandı (her biri için P<0,001) (Tablo 4).

Non-fermenter bakterilerde yatan hastalarda siproflaksasin, imipenem ve meropenem direnci %60'ın üzerinde bulundu. Aminoglikozid antibiyotiklere ayaktan hastalarda direncin ise %25'in altında olduğu saptandı (Tablo 5).

Streptokoklarda penisilin direncinin (%8,4) düşük olduğu saptandı. Stafilokoklarda gentamisinde direnç düşük olup (%5,3), vankomisin ve linezolid direnci saptanmadı (Tablo 6).

Tablo 2. İdrar kültürleri üreme sonuçlarının yıllara ve tedavi gruplarına göre dağılımı

Yıllar	Ayaktan: 12814 (%77,5)				Yatan: 3711 (%22,5)				Toplam
	Gr (-)		Gr (+)		Gr (-)		Gr (+)		
	n	%	n	%	n	%	n	%	n
2022	1511	85,4	259	14,6	408	85,2	71	14,8	2249
2021	1393	85,5	237	14,5	289	85,0	51	15,0	1970
2020	1134	84,8	203	15,2	389	88,0	53	12,0	1779
2019	1130	89,7	130	10,3	342	89,8	39	10,2	1641
2018	1488	64,5	221	9,6	517	22,4	82	3,6	2308
2017	1318	65,9	190	9,5	399	20	93	4,7	2000
2016	1252	67,4	203	10,9	364	19,6	39	2,1	1858
2015	1152	68,4	167	9,9	325	19,3	39	2,3	1683
2014	710	68,5	116	11,2	185	17,8	26	2,5	1037
Toplam	11088	86,5	1726	13,5	3218	86,7	493	13,3	16525
Gr (-): 14306 (% 86,6)					Gr (+): 2219 (%13,4)				

Tablo 3. Tüm izolatlarda ayaktan ve yatan hastalara göre direnç oranları

	AMP		AMC		SXT		CIP		F		CXM		GN		FOS		CRO		ERT	
	A	Y	A	Y	A	Y	A	Y	A	Y	A	Y	A	Y	A	Y	A	Y	A	Y
2022	62,5	79,3	42,2	61	30,5	59,4	42,3	74,2	9,8	30,7	36,6	67,5	10,5	29,8	8,6	21,7	29,7	61,4	2,6	28,7
2021	63,3	82,3	42,6	69,6	34,6	64,3	35,6	79	10,2	30,2	36,2	65,5	11,3	33,7	9,9	19,8	29,3	63	2,3	26,2
2020	62,8	82,8	45,7	70,8	36,5	67,9	33,3	73,9	10,9	44,9	38,8	76,9	11,9	44,6	9,8	24,6	33,5	72,6	2,8	37,5
2019	64,7	84,8	44,5	77,6	32,9	64,8	34,9	71,8	8,2	44,4	39,3	78,8	11,9	44,6	8,3	19,9	33,4	75,9	2,8	40,2
2018	61,5	78,3	31,2	57,8	31,6	55,5	42,9	68,6	10,5	34,5	36,3	72,1	10,8	32,7	7,8	19,4	28,4	63,2	2	23,4
2017	58	79,4	31,5	60,9	33,6	58,3	45,2	67,6	6	21,7	33,3	64,9	13,8	38,3	6,2	19,1	25,4	63,6	2,5	28
2016	59,7	82,7	42	71,3	33,4	57	32,8	63,2	3,7	11,8	37,6	70,3	15,8	37,2	8,7	21,9	22,2	62,1	7,5	33,1
2015	61,9	76,2	3,9	66,5	36,4	47,4	31,3	60,3	7,5	24,5	30,6	65,2	12,4	3	8,1	13,4	24,5	54,9	4,2	23,4
2014			3,3	60,9	36,5	49,7	29,4	51,2	15,1	31,4	28,5	54,7	13,3	26,5			23,5	47,1	4	21,6
Top.	61,8	80,8	33,8	65,8	33,7	58,8	37	68,7	9,1	31,5	35,8	69,7	12,3	32,9	8,5	20,5	27,9	63,3	3,4	29,3
Genel	65,7		40,2		38,9		44,6		13,2		42,4		16,9		10,8		34,5		8,4	
P	<0,001		<0,001		<0,001		<0,001		<0,001		<0,001		<0,001		<0,001		<0,001		<0,001	

AMP= Ampisilin, AMC= Amoksisilin-klavulonik asit, SXT= trimetoprim-sulfametoksazol, CIP= siprofloksasin, F= nitrofurantoin, CXM= sefuroksim, GN= gentamisin, FOS= fosfomisin, CRO= seftriakson, ERT= ertapenem, A= ayakta tedavi gören hastalar, Y= yatarak tedavi gören hastalar, Top= toplam.

Tablo 4. *E. coli* izolatlarında ayaktan ve yatan hastalara göre direnç oranları (%)

	AMP		AMC		SXT		CIP		F		CXM		GN		FOS		CRO		ERT		P
	A	Y	A	Y	A	Y	A	Y	A	Y	A	Y	A	Y	A	Y	A	Y	A	Y	
2022	58,7	75,9	41,2	54,7	31,2	49,7	42,2	61,7	3,6	4,2	37,1	60,7	9,9	14,6	3,8	4,5	29,3	49,2	1,4	10,7	<0,001
2021	59,1	80,3	42,5	66,9	29,1	55,6	38,7	74,1	2,4	5,8	35,3	86,3	10,9	18	2,6	5,1	28,6	52,5	0,8	5,1	<0,001
2020	59,8	78,4	45,8	64	29,8	51,9	31,2	69,2	2,1	6,8	37,2	67,2	10,3	21,5	2,1	4,3	33	60,5	0,6	6,5	<0,001
2019	59,7	80,5	43,5	66,4	31,1	48,7	33,9	66,4	2,2	5,5	37,4	69,9	11,1	22,1	2,3	5,5	32,7	66,4	1	6,2	<0,001
2018	58,4	76,4	30,8	50	32,1	48,3	46,1	63,8	3,8	6,3	33	60,1	10,4	24,5	1,9	3,3	29,5	56,9	0,7	6,4	<0,001
2017	58,3	79,4	30,2	52,5	34	52	43,7	66,1	3,2	6,5	32,3	58	13,3	26,5	2,6	3,5	31,3	57,2	1,3	4,7	<0,001
2016	57,9	77,9	42	66,6	34,1	52,3	31	62,7	2,8	9,2	38	70,3	14,2	30,7	1,8	3,4	21,9	54,6	2,6	9,5	<0,001
2015	61	70,6	34,7	61,5	37,2	46,8	31	61,2	2,6	3,4	30	57,3	10,9	17	3,4	5,9	15,5	47,3	11,6	9	<0,001
2014			31,5	56	38,3	53,1	25	50,4	4,1	10,2	24,8	50	11	16,7			22,8	44,6	2,4	14	<0,001
Top.	58,9	77,7	38,1	58,9	32,7	50,8	36,9	64,1	3	6,2	34,2	64,1	11,3	21,5	2,5	4,2	27,3	54,2	0,6	7,9	<0,001
Gen.	61,5		40,9		35,2		40,6		3,4		38,4		12,7		2,8		31,1		2,5		<0,001

AMP= Ampisilin, AMC= Amoksisilin-klavulonik asit, SXT= trimetoprim-sulfametoksazol, CIP= siprofloksasin, F= nitrofurantoin, CXM= sefuroksim, GN= gentamisin, FOS= fosfomisin, CRO= seftriakson, ERT= ertapenem, A= ayakta tedavi gören hastalar, Y= yatarak tedavi gören hastalar, Top= toplam.

Tablo 5. Non-fermenter bakteri izolatlarında ayaktan ve yatan hastalara göre direnç oranları

	CAZ		TZP		GN		AK		IMP		MEM		FEP		CIP	
	A	Y	A	Y	A	Y	A	Y	A	Y	A	Y	A	Y	A	Y
2022	43,6	76,8	66	87,8	24,2	68,4	18,9	50	42,9	82,2	13,7	60,8	51,7	80,5	80,1	41,1
2021	27,8	73,3	47,1	81,4	9,8	58,1	17,4	50	33,3	83,3	19	67,6	20	22,2	52	81,8
2020	4,3	41	17	50	15,4	40	10,2	30,4	17	61,3	21,3	64,1	14,3	66,7	45,2	62,3
2019	20,6	42,9	22,6	59,2	17,6	38,5	17,6	29,5	11,8	58,9	11,8	63,6	0	100	48	55,8
2018	23,1	33	39,1	47,6	23,6	43,3	14,5	27,2	23,6	65,2	28	67	22,2	31,9	34,5	60
2017	28,5	17	63,3	68,7	45,5	40,4	35,4	36,1	21,4	54,9	15,1	57,1	31,2	34,1	42,4	57,1
2016	14,2	32,6	18,1	30,6	37,8	44,8	29,7	35,5	5,4	37,5	13,5	47,3	13,6	32,6	51,3	51,7
2015	25	47,2	10	56,4	33	50,6	18,1	40,9	18,7	59	12,1	57,3	30	57,1	51,5	66,2
2014	42,3	35	17,2	26,3	44,8	40	17,2	20	10,3	40	17,2	35	41,3	45	55,1	40
Toplam	28,7	49,9	37,6	63,5	24,9	53,0	22,4	40,0	23,1	67,8	19,0	65,0	28,0	58,8	57,5	64,5
Genel	34,3		47,5		32,1		27,2		37,9		34,5		37,2		59,1	

AMP= Ampisilin, AMC= Amoksisilin-klavulonik asit, SXT= trimetoprim-sulfametoksazol, CIP= siprofloksasin, F= nitrofurantoin, CXM= sefuroksim, GN= gentamisin, FOS= fosfomisin, CRO= seftriakson, ERT= ertapenem, A= ayakta tedavi gören hastalar, Y= yatarak tedavi gören hastalar, Top= toplam.

Tablo 6. Gram pozitif bakterilerde direnç oranları

	P	E	DA	VA	CIP	LEV	GN-YDGN	LZD	AMP
Enterokoklar									
Ayaktan	27,7	15,3		3,5	14,2		24,5	1,2	17,7
Yatan	42,5	28,6		6,3	23,3		38,2	2,6	43,2
Genel	31,3	22,2		4,7	18,6		29,9	1,6	27,5
Streptokoklar									
Ayaktan	6,9	17,6		4,6	16,6			1,5	
Yatan	10,6	32,3		7,2	22,3			4,0	
Genel	8,4	23,3		5,4	19,3			2,4	
Stafilokoklar									
Ayaktan	68,3	53,6	16,8	0	23,2	17,5	3,5	0	
Yatan	77,5	62,5	22,3	0	29,3	25,6	7,63	0	
Genel	71,2	55,8	19,2	0	25,6	19,5	5,3	0	

Gentamisine direnç oranları enterokoklarda yüksek düzey gentamisin direnci olarak verilmiştir. P= penisilin, E= eritromisin, DA= klindamisin, Va= vankomisin, CIP= siprofloksasin, LEV= levofloksasin, GN= gentamisin, YDGN= yüksek düzey gentamisin, LZD= linezolid, AMP= ampisilin.

4. Tartışma ve Sonuç

İdrar yolu enfeksiyonu olgularında etken mikroorganizmanın ve antibiyotik duyarlılık testinin belirlenmesi tedavi yönetiminin belirlenmesi açısından kritik öneme sahiptir. Ancak bu süreçte tedavinin ampirik olarak başlanması gerekmektedir. Bu açıdan genel olarak İYE olgularında etkenleri ve genel antibiyotik duyarlılık profillerinin güncel olarak bilinmesi ampirik tedavi seçeneklerinin daha doğru olarak belirlenmesine katkı sağlar (Hooton, 2012; Bader ve ark., 2017; Caron ve ark., 2018; Warzecha ve ark., 2021). Bu çalışmada artan direnç oranlarına rağmen ampirik tedavi için fosfomisin ve nitrofurantoinin hala iyi seçenekler olduğu görülmüştür.

Yapılan çalışmalarda İYE olgularında en sık saptanan etkenlerin *E. coli*, *Klebsiella pneumoniae* ve *Proteus mirabilis* olduğu gösterilmiştir (Meena ve ark., 2021; Islam ve ark., 2022; Prasada Rao ve ark., 2022). Çalışmamızda etkenlerin %86,6'sının gram (-) bakteri ve %62,9'unun *E. coli* olduğu saptanmıştır. Bu bulgular İYE olgularında hala en sık etkenlerin Gram (-) enterik bakteriler olduğunu ve *E. coli*'nin hala olguların çoğunda etken olarak izole edildiğini göstermektedir.

Siprofloksasin uzun süre İYE olgularında ampirik tedavide ilk seçenekler arasında yer almıştır. Ancak direnç oranlarının hızla artması siprofloksasinin bu olgularda kullanımını sınırlamıştır. Ancak siprofloksasin İYE olgularının ampirik tedavisinde hala tercih edilen antimikrobiyallerden biridir (Meena ve ark., 2021; Prasada Rao ve ark., 2022; Islam ve ark., 2022; Sherchan ve ark., 2022; Salh, 2022; Majumder ve ark., 2022). Son yıllarda yapılan bazı çalışmalarda Gram (-) enterik bakterilerde siprofloksasine direnç oranlarının %22-85 aralığında olduğu bildirilmiştir (Meena ve ark., 2021; Prasada Rao ve ark., 2022; Islam ve ark., 2022; Sherchan ve ark., 2022; Salh, 2022; Majumder ve ark., 2022). Salh (2022) Gram (-) bakteri kaynaklı İYE olgularında nitrofurantoinin hala etkili olduğunu rapor etmişlerdir. Ballén ve ark. (2021) İYE olgularında *K. pneumoniae* izolatlarında fosfomisine direnç oranının %23 olduğunu bildirmişlerdir. Ülkemizde son yıllarda yapılan çalışmalarda toplum kaynaklı İYE olgularında Gram negatif bakteri izolatlarında fosfomisine direnç oranını %1-2 olarak, siprofloksasine direnç oranını %23-45 olarak bildirilmiştir (Seno ve ark., 2020; Keskin ve ark., 2021). Çalışmamızda ayakta tedavi gören hastalarda siprofloksasin direncinin yüksek ve artmakta olduğu (%44,6); fosfomisin (%10,8) ve nitrofurantoin (%13,2)

dirençlerinin düşük olduğu görülmüştür. Bu bulgular ayaktan tedavi gören hastalarda fosfomisin ve nitrofurantoinin iyi birer alternatif olmaya devam ettiğini ancak siprofloksasin tedavisine yanıtın izlenmesi gerektiğini göstermektedir.

Komplike İYE olgularında, yatan hastalarda ya da hastane kaynaklı İYE olgularında seftriakson ve ertapenem sıklıkla tercih edilen ampirik tedavi ajanlarındandır (Caron ve ark., 2018; Warzecha ve ark., 2021; Abbott ve ark., 2022). Islam ve ark. *E. coli* izolatlarında siprofloksasine direnç oranını %73 olarak bildirmişlerdir (Islam ve ark., 2022). Majumder ve ark. (2022) İYE olgularında izole edilen Gram (-) izolatlarda seftriaksona direnç oranını genel olarak %64,5 olarak saptamışlardır. Çalışmamızda yatan hastalarda intravenöz olarak tercih edilen seftriakson direncinin (%63,3) yüksek olduğu, ertapenem direncinin (%29,3) daha düşük olduğu görüldü. Bu bulgular yatan hastalarda seftriakson tedavisine yanıtın yakından izlenmesi gerektiğini ve ampirik tedavi için uygunluğunun tartışılabilir olduğunu, ertapenemin hala iyi bir alternatif olarak tedavi seçenekleri arasında yer aldığını ancak direnç oranlarının izlenmesi gerektiğini göstermektedir.

Toplum kaynaklı İYE olgularında nitrofurantoin uzun zamandır ampirik tedavide kullanılmaktadır. Fosfomisin ise bu olgularda son yıllarda ilk seçilen antibiyotiklerden biri olarak kullanılmaya başlamıştır. Ertapenem daha çok yatan hastalarda ve tedaviye dirençli olgularda kullanılmaya başlanan antimikrobiyallerdendir (Sastry ve Doi, 2016; Chu ve Lowder, 2018; Abbott ve ark., 2022). Prasada Rao ve ark. (2022) nitrofurantoine direnç oranını İYE olgularından soyutlanan Klebsiella izolatlarında %0; *E. coli* izolatlarında %28,9; *Pseudomas aeruginosa* izolatlarında %67,8 olarak bulmuşlardır. Haindongo ve ark. (2022) nitrofurantoin direnç oranını *E. coli* izolatlarında %12,4; Klebsiella izolatlarında %23,2 olarak bildirmişlerdir. Majumder ve ark. (2022) İYE olgularında izole edilen Gram (-) etkenlerde nitrofurantoine direnç oranını %25,4 olarak saptamışlardır. Ülkemizde son yıllarda yapılan çalışmalarda toplum kaynaklı İYE olgularında Gram negatif bakteri izolatlarında nitrofurantoine direnç oranını %2-4 olarak, ertapeneme direnç oranını %5 olarak bildirilmiştir (Seno ve ark., 2020; Keskin ve ark., 2021). Çalışmamızda ayakta tedavi gören ve yatan hastalarda üreyen *E. coli* izolatlarında nitrofurantoin, fosfomisin ve ertapenem dirençlerinin %10'un altında olduğu saptanmıştır. Bu bulgular ampirik tedavide nitrofurantoin, fosfomisin ya da ertapenem tedavisine başlanan olgularda kültür sonucunun yakından takip edilmesi gerektiğini göstermektedir. Çalışmamızda ayrıca *E. coli* izolatlarında tüm antibiyotiklere direnç oranının yatan hastalarda ayakta tedavi görenlere göre anlamlı yüksek olduğu bulunmuştur. Bu bulgu özellikle yatan hastalarda ampirik tedaviye başlarken antibiyotik seçiminin genel direnç profilinin bilinerek yapılması gerekliliğini ortaya koymaktadır.

Non-fermenter bakteriler hastane kaynaklı İYE olgularında etken olarak sıklıkla izole edilmektedir. Bu izolatlarda İYE olgularının ampirik tedavisinde sık kullanılan antibiyotiklere çoğunlukla direnç görülmektedir. Bu nedenle ampirik tedavi seçenekleri Gram (-) enterik bakteri kaynaklı olgulara göre çok daha kısıtlıdır (Caron ve ark., 2018; Seno ve ark., 2020; Keskin ve ark., 2021; Prasada Rao ve ark., 2022; Majumder ve ark., 2022). Prasada Rao ve ark. (2022) siprofloksasine direnç oranını İYE olgularından soyutlanan *P. aeruginosa* izolatlarında %17,6 olarak bulmuşlardır. Majumder ve ark. İYE olgularında izole edilen *P. aeruginosa* suşlarında karbapenemlere direnç oranını %17-23 arasında, siprofloksasine direnç oranını ise %67 olarak bulmuşlardır (Majumder ve ark., 2022). Ülkemizde ise Keskin ve ark. (2021) *P. aeruginosa* izolatlarında siprofloksasine direnç oranını %43 olarak bildirmişlerdir. Çalışmamızda yatan hastalarda non-fermenter izolatlarda siproflaksasin, imipenem ve meropenem direnci %60'ın üzerinde bulunmuştur. Bu bulgular kültür sonucunun özellikle yatan hastalarda önemini ve bu hastalarda özellikle ampirik olarak karbapenem başlanan olgularda kültür sonucunun izlenmesinin önemli olduğunu göstermektedir.

Gram (+) etkenler İYE olgularında büyük sıklıkta izole edilmemektedir (Seno ve ark., 2020; Sherchan ve ark., 2022; Majumder ve ark., 2022). Shercan ve ark. (2022) İYE olgularında izole edilen Gram (+) etkenlerde vankomisin ve linezolide dirençli suş saptamamışlardır. Majumder ve ark. (2022) ise Gram (+) izolatlarda gentamisine direnç oranının %29-53 aralığında olduğunu bildirmişler, vankomisin ve linezolide dirençli suş saptamamışlardır. Ülkemizde ise Keskin ve ark. (2021) Gram pozitif izolatlarda vankomisin ve linezolide dirençli suş saptamamışlardır. Çalışmamızda Gram pozitif bakterilerde streptokoklarda penisilin (%8,4) direnci düşüktür. Stafilokoklarda gentamisine direnç düşük olup (%5,3), vankomisin ve linezolid direnci saptanmadı. Bu bulgular Gram pozitif bakteri kaynaklı olgularda ampirik tedavi seçeneklerinin hala geniş olduğunu göstermektedir. Ancak vankomisin ve linezolid gibi bazı antibiyotiklerin Gram negatif bakterilere etkisiz olduğu ve idrar yolu enfeksiyonu olgularında çoğunlukla Gram negatif etkenlerin izole edildiği gibi faktörler birlikte değerlendirildiğinde bu antibiyotiklerin bu olgularda ampirik tedavi için uygun seçenekler arasında olmadığı görülmektedir.

Çalışmamızda bazı kısıtlamalar yer almaktaydı. Çalışmamız geriye dönük ve kesitsel olarak planlandığından ve hastaların çoğunun kontrol muayenesine gelmemesi ve kontrol kültürlerinin yapılmamış olması dolayısıyla ampirik tedavi sonuçlarına ulaşılamamıştır. Ayrıca hastalar uzun dönemde izlenmemiştir. Bu nedenlerle istatistiksel hata olasılığını düşürmek amacıyla çalışmaya dahil edilen hasta sayısı yüksek tutulmuştur.

Çalışmamızda elde edilen bulgular kültür imkanı olmayan durumlarda veya kültür antibiyogram sonuçları elde edilene kadar ayaktan tedavide nitrofurantoin ve

fosfomisinin, yatan hastalarda ertapenem ve fosfomisinin diğer antibiyotiklere göre ampirik tedavide başarılı olabileceğini göstermektedir.

Katkı Oranı Beyanı

Yazar(lar)ın katkı yüzdesi aşağıda verilmiştir. Tüm yazarlar makaleyi incelemiş ve onaylamıştır.

	M.Ö.	Z.Ş.K.
K	50	50
T	50	50
Y	50	50
VTI	80	20
VAY	80	20
KT	60	40
YZ	60	40
KI	50	50
GR	50	50
PY	50	50
FA	50	50

K= kavram, T= tasarım, Y= yönetim, VTI= veri toplama ve/veya işleme, VAY= veri analizi ve/veya yorumlama, KT= kaynak tarama, YZ= Yazım, KI= kritik inceleme, GR= gönderim ve revizyon, PY= proje yönetimi, FA= fon alımı.

Çatışma Beyanı

Yazarlar bu çalışmada hiçbir çıkar ilişkisi olmadığını beyan etmektedirler.

Etik Onay/Hasta Onamı

Bu çalışma yerel etik kurul, Samsun Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından onaylandı (onay tarihi: 13 Temmuz 2023, onay numarası: SÜKAEK-2023 7/13) ve retrospektif olarak planlandı. Araştırma Helsinki Deklarasyonu Prensipleri'ne uygun şekilde yapılmıştır.

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EVALUATION OF COLORECTAL CANCER SCREENING AWARENESS AND COMPLIANCE RATES OVER THE AGE OF 40: SINGLE CENTER DATA IN TÜRKİYE

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Abstract: The frequency of colorectal cancer is increasing under the age of 50, and new sights have emerged regarding the initiation of screening in the earlier age group. This study aims to measure the consciousness of patients over the age of 40 about colorectal cancer screening and to reveal the screening rates of individuals over the age of 50. 300 consecutive patients who applied to our center and were older than 40 years of age were included. The questionnaire was created based on current guidelines and literature knowledge by the investigators and done through face-to-face interviews. Patients over 50 years of age who did not undergo screening were analyzed by dividing them into groups according to their socio-demographic characteristics and colorectal cancer risk factors. While 64.7% of the participants stated that they knew about colorectal cancer screening, only 32.4% of the participants aged 50 and over had colorectal cancer screening. The vast majority of patients stated that they did not have enough knowledge about the subject. The rate of participants who stated that they were considering entering the cancer screening program after completing this questionnaire was 73.7%. Colorectal cancer screening rates of non-smokers, women, and married participants were higher than the other group (P=0.016, P=0.017, and P=0.033, respectively). This study shows low screening compliance of individuals over the age of 50. We think that it is important to encourage and inform all adults over the age of 40 about colorectal cancer screening for public health.

Keywords: Cancer screening, Colon cancer, Colorectal cancer, Rectum cancer, Risk factors

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1. Introduction

Colorectal cancer is the 4th most common cancer among adults worldwide. However, it ranks 3rd in mortality after lung and breast cancer (WHO, 2022). Considering the course of the last 20 years, it is observed that although the incidence has decreased, its place among the causes of mortality has not changed. Again, in the last 20 years, a significant increase has been revealed in the number of people diagnosed with colorectal cancer aged 50 and younger (Stoffel and Murphy, 2020). In a study conducted with a large cohort of patients younger than 50 years of age who were diagnosed with colorectal cancer without any risk factors, it was shown that 86% of this patient group were symptomatic at the time of admission and were mostly diagnosed at an advanced stage (Dozois et al., 2008). The most common risk factors for colorectal cancer are a family history of colorectal cancer in first-degree relatives, hereditary polyposis syndromes, and a personal history of adenomatous polyp, a history of inflammatory bowel disease, obesity, and smoking. However, even if there are no risk factors, it is recommended that all individuals over the age of 50 should be included in the screening program for colorectal cancer (Lansdorp-Vogelaar and Von Karsa, 2012). The goal of cancer screening programs is to reduce disease-specific mortality through early diagnosis. Colon, breast, and prostate cancer screenings have shown that specific mortality for these cancers decreases (Cronin et al., 2018).

Colorectal cancer screening methods are stool occult blood examination once a year, sigmoidoscopy, or total colonoscopy every 10 years starting from the age of 50 (Lansdorp-Vogelaar and Von Karsa, 2012). Since sigmoidoscopy and total colonoscopy are interventional tests, different methods such as computed tomography colonography, stool DNA test, and capsule endoscopy are also being developed for colorectal cancer screening, but their routine use in colorectal cancer screening is not recommended yet (Lansdorp-Vogelaar and Von Karsa, 2012). In Türkiye, all men and women between the ages of 50-70 are screened for colorectal cancer by performing a stool occult blood test every two years and a colonoscopy every 10 years. Colorectal cancer screening is terminated in 70-year-old individuals who have negative occult blood tests in the last two stools (URL1).

Since the frequency of colorectal cancer diagnosis is increasing under the age of 50 and it is diagnosed at a more advanced stage in this age group, new opinions have emerged regarding the initiation of screening in the earlier age group. Accordingly, colorectal cancer screening is recommended for all adults aged 50-75 with evidence level A, and adults aged 45-50 with evidence level B. It is recommended to screen according to the clinician's evaluation in the group over 75 years old (Davidson et al., 2021).

This study aims to measure the level of knowledge about colorectal cancer screening of patients over the age of 40 who applied to the Internal Medicine outpatient clinic of Samsun Training and Research Hospital and to reveal the screening rates of individuals over the age of 50.

2. Materials and Methods

300 consecutive patients over the age of 40 who applied to Samsun Training and Research Hospital Internal Medicine outpatient clinic were included in the study. The participants were asked whether they had information about colorectal cancer screening programs, from which sources they accessed information about colorectal cancer screening methods, and whether they found the information provided on this subject sufficient. The colorectal cancer screening histories of the participants were questioned, and patients who did not undergo screening were analyzed for reasons. In addition, the participants were questioned in terms of colorectal cancer risk factors and it was investigated whether there was a difference in colorectal cancer screening behavior between people who were in the risk group and those who were not. To reach all these data, the questionnaire form was created by the researchers and filled out face-to-face for each participant. An informed consent form was filled out by all participants. The questions asked in the study form are listed in Table 1.

Table 1. The questionnaire form

1.Demographical properties	
Name/ surname	
Age	
Date of birth	
Employment status	
Educational status	
Marital status	Divorced/widow
	Married
	Single
Having child	Yes
	No
2. Questioning Risk Factors for Colorectal Cancer	
Do you smoke?	Yes
	No
Is there a family history of colorectal cancer in first degree relatives?	Yes No
Is there a family history of other cancer in the first degree relative?	Yes No
If yes, please specify which cancer.	
Have you ever had a bowel disease?	Yes No
If your answer is yes, please state the name of your disease.	Bowel Polyp Ulcerative Colitis Crohn's Disease Hemorrhoids Irritable Bowel Syndrome Other (Please Specify)
Height and Weight	
3. Awareness Level Survey on Colorectal Cancer Screening Program	
Do you know that early diagnosis can be made by screening for colorectal cancer?	Yes No
Could you please indicate which of the following cancer screening methods you have heard of and have knowledge of? You can tick more than one option	Stool occult blood test once a year Sigmoidoscopy examination once every 5 years Total colonoscopy method once in 10 years
Have you heard of the name “Cancer Early Diagnosis, Screening and Education Center (KETEM)” before?	Yes No
From which source did you learn about colorectal cancer screening? You can tick more than one option.	From the family doctor From an internist From a general surgeon From a gastroenterologist From KETEM posters From the media organs From my neighbors, my family, my surroundings Other (Please specify.)
Do you think the information about colorectal cancer screening is sufficient?	Yes No
At which age do you think colorectal cancer screening begins?	over 40 over 45 years old over 50 over 60 years old
Do you think colorectal cancer screening is a paid service?	It is paid It is not paid
4. Colorectal cancer screening information	
When was the last time you had a stool occult blood test?	In the last 1 year more than 1 year ago I have never done
If you have never done it, could you please indicate why?	I don't think I'm in the appropriate age range for this screening program I am not familiar with this scanning program. I did not have it done because I was afraid to give a stool test.
When was the last time you had a sigmoidoscopy or colonoscopy?	I have done it in the last 5 years I've had it done in the last 10 years I have never done
If you have never done it, could	I don't think I'm in the

you please indicate why?		appropriate age range for this screening program I am not familiar with this scanning program. I did not have it done because I was afraid to have a colonoscopy
After completing this questionnaire, would you consider making a KETEM appointment and entering the cancer screening program?		Yes No

KETEM= Cancer early diagnosis, screening and education center.

This study was evaluated by the Ethics Committee of Samsun Training and Research Hospital and approved ethically with the number GOKA/2021/15/2. The Declaration of Helsinki was complied with in the study.

2.1. Statistical Analysis

Study data were analyzed using the SPSS package program, Version 22 (IBM Corp, 2013). Normally distributed variables were expressed as mean ±standard deviation and non-normally distributed variables were expressed as median (lowest-highest). Chi-square and Fisher's exact tests were used to compare categorical variables between groups. A P-value below 0.05 was considered statistically significant.

3. Results

A total of 300 patients, 167 women and 133 men, who applied to the Internal Medicine outpatient clinic of Samsun Training and Research Hospital were included in the study. The mean age was 59.5 ± 12.2 years. The mean body mass index was 29.1±5.1 kg/m^2. Demographic data are summarized in Table 2.

The analysis and results of the questions evaluating the knowledge and awareness of the people participating in the study about colorectal cancer screening are summarized in Table 3.

Table 2. Demographics

Demographic data		N (%)
Gender	Female	167 (55.7)
	Male	133 (44.3)
Educational status	Not literate	40 (13.3)
	Primary school graduate	166 (55.3)
	Secondary school graduate	23(7.7)
	High school graduate	33 (11.0)
	Graduated from a university	33 (11.0)
	Higher education after university	5 (1.7)
Employment status	Active employer	85 (28.3)
	Not working	134 (44.7)
	Retired	81 (27.0)
Marital status	Married	257(85.7)
	Single	9 (3)
	Widow	34 (11.3)
Having a child	Yes	281 (93.7)
	No	19 (6.3)

Table 3. Knowledge level about colorectal cancer screening

	Yes n (%)	No n (%)
Did you know that early diagnosis can be made by screening for colorectal cancer?	194 (64.7)	106 (35.3)
Have you ever heard of the screening method with stool occult blood test once a year?	81 (27.0)	219 (73.0)
Have you ever heard of the screening method with sigmoidoscopy examination once in 5 years?	22 (7.3)	278(92.7)
Have you heard of the total colonoscopy method once in 10 years?	76 (25.3)	224 (74.7)
Have you ever heard of cancer early detection, screening and education centers (KETEM)?	191(63.7)	109 (36.3)
Do you think the information about colorectal cancer screening sufficient?	117(39.0)	183 (61.0)
Do you think colorectal cancer screening is a paid service?	24 (8.0)	276 (92)
From which source did you learn about colorectal cancer screening?	n (%)	
a. From my family doctor	59 (30.4)	
b. From an internist	43 (22.2)	
c. From a general surgeon	17 (8.8)	
d. From a gastroenterologist	19 (9.8)	
e. KETEM posters	37 (19.1)	
f. From press organs	53 (27.3)	
g. From my neighbors, my family, my surroundings	68 (35.1)	
h. Other	11 (5.7)	

While 64.7% of the patients stated that they knew that colorectal cancer could be diagnosed early by applying screening methods, it was seen that stool occult blood tests and colonoscopy were known at a similar rate. “Do you find the information about colorectal cancer screening sufficient?” It was seen that 61% of the participants answered “no” to the question. “After completing this questionnaire, would you consider making an appointment with KETEM and entering the cancer screening program?” 73.7% of the participants answered “yes” to the question.

Since the national cancer screening program recommends screening for colorectal cancer over the age of 50, the cancer screening rates of patients for this group were evaluated. 222 (74%) of the participants were 50 years and older. It was determined that 72 (32.4%) of these 222 people had colorectal cancer screening by any method. It was determined that 173 (77.9%) had never had a stool occult blood test, and 153 (68.9%) of them ticked the option “I do not know about this screening

program". It was determined that 177 (79.7%) participants had never had a sigmoidoscopy or colonoscopy, and 150 (67.6%) of them choose the option "I do not know about this screening program".

When the general colorectal cancer screening behavior over the age of 50 is analyzed according to gender, educational status, employment status, having a child, marital status, family history of colorectal cancer or another cancer, there is a statistically significant difference between women compared to men and non-smokers compared to smokers. It was found that they had more scans. It was also revealed that married people were more likely to comply with colorectal cancer screening than those living alone. Colorectal cancer screening was found to be significantly higher in first-degree relatives with a history of cancer and in those with a history of bowel disease (Table 4).

Table 4. The effect of risk factors and demographic characteristics on cancer screening behavior in patients over 50 years of age

	Screened for Cancer	Not screened for Cancer	P-value*
	n (%)	n (%)	
With at least one risk factor	44 (32.4)	92 (67.6)	
No risk factor	28 (32.6)	58 (67.4)	0.975
Smoker	4 (13.3)	26 (86.7)	
Non-smoker	68 (35.4)	124 (65.5)	0.016
Obese	34 (34.0)	66 (66.0)	
Normal weight	38 (31.1)	84 (68.9)	0.651
There is a family history of colorectal cancer in a first-degree relative	10 (38.5)	16 (61.5)	
There is not family history of colorectal cancer in a first-degree relative	62 (31.6)	134 (68.4)	0.485
There is a family history of any cancer in a first-degree relative	32 (43.2)	42 (56.8)	
There is not family history of any cancer in a first-degree relative	40 (27.0)	108 (73.0)	0.015
History of bowel disease	19 (52.8)	17 (47.2)	
Not a history of bowel disease	53 (28.5)	133 (71.5)	0.004
Gender			
Female	45 (39.8)	68 (60.2)	
Male	27 (24.8)	82 (75.2)	0.017
Marital status			
Married	56 (29.6)	133 (70.4)	
Single or widow	16 (48.5)	17 (51.5)	0.033
Educational status			
Secondary school and below	53 (31.4)	116 (68.6)	
High school and more	19 (35.8)	34 (64.2)	0.543

* Data of 222 patients over 50 years of age were analyzed with the chi-square test.

4. Discussion

It has been accepted that a routine screening program should be applied to diagnose colorectal cancer early and to prevent mortality due to this disease. According to both the United States and European guidelines, screening for colorectal cancer is recommended for every adult from the age of 50 (Lansdorp-Vogelaar and Von Karsa, 2012; Cronin et al., 2018; Davidson et al., 2021). It is recommended that individuals with high risk such as familial polyposis be screened starting from an earlier age and following certain algorithms. In our country, colorectal cancer screening is carried out through the Cancer Early Diagnosis, Screening, and Education Center (KETEM). Again, screening for colorectal cancer with stool occult blood is recommended for individuals over the age of 50 who are followed up in family health centers. This study, it was aimed to measure the general knowledge levels and tendencies of patients over the age of 40 who applied to the Internal Medicine clinic about colorectal cancer screening. The reason for choosing the age of 40 as the limit in our study is that there are opinions on screening can start at an earlier age due to the increasing number of young colorectal cancer cases (Dozois et al., 2008).

In our study, although the rate of those who answered "yes" to the question "Do you know that early diagnosis can be made by screening for colorectal cancer?" was 64.7%, the rate of those who knew stool occult blood test and colonoscopy was found to be low (27% and 25.3%, respectively). Moreover, the sample set for this study consisted of adults over the age of 40, and nearly two-thirds of the participants were over the age of 50. This

suggests that the patients who applied to our center for colorectal cancer screening do not have enough information. The rate of those who had general knowledge about KETEM was 63.7%.

In our study, there were 222 people over the age of 50. It was determined that only 72 (32.4%) of these 222 people had colorectal cancer screening by any method. It was determined that 173 (77.9%) of them had never had a stool occult blood test and 177 (79.7%) of the participants had never had a sigmoidoscopy or colonoscopy. In a study in which breast, colon, and cervical cancer screening rates were questioned in Türkiye, the rate of having at least one mammography over the age of 50 was 48.2%, and the rate of having at least one colonoscopy over the age of 50 was 12%. In the same study, it was revealed that having knowledge about the national cancer screening program and knowing where the cancer screening was carried out was effective in screening behavior (Gulten et al., 2012).

In a study examining the effect of health literacy on cancer screening behavior, it was revealed that as health literacy increases, the tendency to not screen for colorectal cancer increases10. In the same study, the colorectal cancer screening rates in the general population were also found to be 21.1%, which was found to be consistent with our study (Pancar and Mercan, 2021). In a recently published study by Tekiner et al. (2021) indicated that the colorectal cancer screening attitude of adults between the ages of 18 and 50 was investigated and it was determined that 58.1% of the participants answered "yes" to the statement “a colonoscopy should be performed every 10 years over the age of 50”. Here, the effects of sociodemographic characteristics such as educational status, marital status, and employment status on colon cancer screening behavior were examined. In this study, whose average age was younger (35.4 years), it was observed that people were informed about the subject, and their opinions were asked. In our study, it is questioned how much information people have about the screening program. When the results of both studies are interpreted together, the importance of informing people close to the screening program becomes evident.

In another recent study, the effect of informing the participants about the screening issue and reminding them was investigated, and it was revealed that the reminded group had more compliance with the screening program (Ahmed et al., 2023). In the same study, when married and unmarried (separated, divorced, or widowed) were evaluated in terms of compliance with the screening program, it was determined that the second group had lower screening compliance. In our study, similar to the aforementioned study, it was found that married people were more likely to comply with the colorectal cancer program.

Although cancer screening has become widespread in developed countries, it has been shown that socioeconomic level affects the rates of reaching screening methods and having cancer screening (Sicsic and Franc, 2014; McCowan et al., 2019; Brown et al., 2021). In a study conducted in Japan, it was shown that those who do not regularly work in an active job participate in cancer screenings at a lower level than those who work regularly, and this is especially evident for colorectal cancer screening (Ishii et al., 2023). In our study, the group that is actively employed or retired and has government insurance and the group that does not have a regular working life were compared in terms of compliance with colorectal cancer screening, and no statistically significant difference was found between the two groups (P=0.223). However, the fact that the insurance status of the non-working group was not questioned is among the limitations of our study. For this reason, we think that there is a need for new studies that will investigate the effect of active employment or active insurance status on cancer screening behavior in Türkiye.

In a study investigating the level of colorectal cancer screening among general surgery specialists in our country, it was found that 74 of 156 general surgeons over 50 years of age had colorectal cancer screening, preferably with colonoscopy. In other words, even among the physicians of a branch that is so closely related to the diagnosis and treatment of colorectal cancer, more than half of them do not comply with the screening program themselves (Celik et al., 2019).

Türkiye is among the countries where the incidence of colorectal cancer according to age increases gradually under the age of 50, and the incidence of colorectal cancer under the age of 50 in Türkiye has been shown as 5.9 per 100000 (Globocan, 2020). Despite this, in a study conducted in our country, the awareness of colorectal cancer screening was found to be 25.4% (Zafer et al., 2017). In our study, both colorectal cancer screening awareness and cancer screening rates were found to be low in line with the literature.

We believe in that the results we obtained in this study are important because the level of knowledge of people in the age range close to the colorectal cancer screening program was evaluated. It was determined that the most obvious reason for the patients in this group not to be screened was their insufficient knowledge about the subject. This finding suggests that the announcement and information of the national colorectal cancer screening program should be done more effectively. It was seen that 61% of the participants answered “no” to the question

“Do you think that the information about colorectal cancer screening is sufficient?”. 73.7% of the participants answered “yes” to the question “After completing this questionnaire, would you consider making an appointment with KETEM and entering the cancer screening program?”.

We think that it is important to question colorectal cancer screening information and to encourage patients for colonoscopies, especially patients over 50 years of

age who apply to the internal medicine outpatient clinic for other reasons. Because among the participants, 68.9% of those who did not have stool occult blood tests stated that they did not have this procedure because they did not know, while this rate was 67.6% in those who did not have a colonoscopy. 8.6% of the participants stated that they did not have a colonoscopy done because they were afraid of the procedure. We think that the guidance and support of the physician will help overcome this barrier.

The most important limitation of our study is that the adequacy of the number of participants to represent the population has not been demonstrated. However, all individuals over the age of 40 who applied to our center for 3 months and agreed to fill out the form were accepted. When we look at the demographic characteristics, it is observed that a homogeneous group is formed in terms of age, marital status, employment status, and risk factors. Again, the rates of screening over the age of 50 that we obtained were found to be compatible with the literature. Therefore, we think that the findings represent general population data.

5. Conclusion

This study reveals that patients over the age of 40 who applied to our center do not have enough information about the colorectal cancer screening program, and the participation of individuals over the age of 50 in the cancer screening program is very low. We think that it is important to encourage all adults over the age of 50 who apply to the family medicine and internal medicine outpatient clinics to the colorectal cancer screening program and to inform individuals over the age of 40 about colorectal cancer screening at least once.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	D.S.K.Ö.	E.D.E.	A.U.E.	M.D.D.
C	100			
D	100			
S	100			
DCP		50	50	
DAI	50			50
L	25	25	25	25
W	25	25	25	25
CR	60	10	10	20
SR	25	25	25	25
PM	25	25	25	25

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Approval/Informed Consent

This study was approved by Ethics Committee of Samsun Training and Research Hospital (approval date: February 15, 2021, protocol code: GOKA/2021/15/2).

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HOW DO BIRTH SATISFACTION, PERCEIVED STRESS, AND SOME FACTORS AFFECT THE RISK OF POSTPARTUM DEPRESSION?

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Abstract: This research was carried out to determine the effects of birth satisfaction, perceived stress level and some factors on postpartum depression (PPD). In the study; cross-sectional, descriptive, correlational and predictive research designs were used. 446 women who met the inclusion criteria participated in the study. The average age of the women was 28.48±57.14 (min-max: 19-44). While 13.9% of women were at risk of PPD and there was a negative and moderate relationship between birth satisfaction and PPD risk (r=-0.403), a negative but low-level significant relationship was obtained between perceived stress (r=-0.325). Among the socio-demographic variables, the education level has the highest impact, while among the obstetric characteristics, the disease status of the baby has the highest impact. Perceived stress has the greatest effect compared to all variables. In this study risk of PPD; It was determined that birth satisfaction had a negative effect and perceived stress had a positive effect. Among the socio-demographic characteristics; age (35 years and above), education level; place of residence, obstetric characteristics; It was concluded that pregnancy planning, pregnancy problems, baby's health, pregnancy follow-up and birth support factors are effective on the risk of PPD.

Keywords: Birth, Satisfaction, Stress, Postpartum depression, Risk, Midwife

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1. Introduction

Birth satisfaction defined as the pregnant woman being ready for birth, being respected, communicating effectively, and using methods to cope with pain, uninterrupted support, and giving birth in an appropriate position with minimum obstetric intervention. Satisfaction with birth is an important indicator in evaluating the birth experience. Determining birth satisfaction is important as it is an indicator of the quality of maternal care as well as showing the well-being of the newborn and the mother (Dağlı et al., 2023). Having a positive birth experience; It is emphasized that, in addition to increasing women's self-confidence, it will help them establish stronger relationships with their babies/children and contribute positively to their future birth planning (Reyhan et al., 2023; Dağlı et al., 2023). On the other hand, the birth experience is negative; It has been reported that it increases subsequent pregnancy and birth complications, negatively affects newborn health, causes premature birth, low birth weight, intrauterine growth retardation, especially postpartum depression (PPD) (Hain et al., 2016).

Besides birth satisfaction, stress is also an important factor that can cause PPD. A study in the literature reported that increased stress levels lead to the risk of developing PPD (Scheyer and Urizar, 2016). In addition, Yim et al. stated in their systematic review that the role of stress is important in the development of PPD (Yim et al., 2015). In addition, obstetric characteristics such as number of births, type of birth, and positive psychiatric history have been reported to be effective in the development of PPD (Galiano et al., 2019). The transition to a new life with the birth of a baby requires adjustments in many areas (such as financial, familial, physiological). These regulations can negatively affect the mother's health and increase her stress. Improving maternal health is a costly public health challenge, and the postpartum period deserves further efforts to understand factors that may impair maternal health (Cheng et al., 2009).

Postpartum depression (PPD) is a highly prevalent, debilitating mental disorder. In psychiatric diagnostic systems (DSM-V), PPD is redefined as "peripartum-onset depressive disorder" (APA, 2022). Mothers may feel helpless in the face of situations such as biological changes, genetic vulnerability, environmental stress or inadequate social support associated with motherhood blues. This situation may increase susceptibility to depression. When peripartum mortality rates are examined, suicide rates due to PPD; It was found to be greater than the mortality rates due to postpartum hemorrhage and hypertensive diseases. The postpartum period is the period when women are at the highest risk of developing psychiatric diseases, especially PPD (Dağlı

et al., 2021). 17% in Japan, 23.2% in China, 17.8% in the United Arab Emirates, 1% in Iceland 14, PPD prevalence was reported as 12.7% in Sweden, 12% in England, 6.5-12.9% in Norway, and 15.5% in Sri Lanka (Ahmed et al., 2021; Qi et al., 2021). In studies conducted in Turkey, the frequency of PPD is within a broad perspective of 23.8-61.8% (Ahmed et al., 2021; Dağlı et al., 2021). Therefore, it would be meaningful to examine birth satisfaction specifically for Turkish women.

Once it is determined which PPD risk factors women enter the postpartum period with, these women can be monitored more closely and the development of PPD can be prevented or intervened in the early period. A good understanding of risk factors for PPD will facilitate prevention and screening. In this way, diagnosis can be increased and treatment rates can be improved in high-risk women (Dağlı et al., 2021).

2. Materials and Methods

2.1. Purpose and Design

Current research; It was carried out to determine the effects of birth satisfaction, perceived stress level and other risk factors on PPD. In the study; Cross-sectional, descriptive, correlational and predictive research designs were used. The study was written according to the STROBE checklist.

2.2. Location, Time and Participants of the Research

It was conducted between June 2022 and May 2023 with women giving birth at a university hospital in a city south of Turkey. During this period, 7141 women gave birth in this hospital. The sample size of the study was determined with the G Power program. Urbanová et al. (2021) study, it was calculated that at least 412 women should be included in this study when the effect size was determined as d = 0.15, the power of the test was determined as p = 0.95, and the confidence interval was 90%. the margin of error was 5% (Urbanova et al., 2021). 446 women were included in the study. Inclusion criteria; Women two to four days postpartum were postpartum women who were past 37 weeks of gestation, had a healthy baby, were healthy, and volunteered to participate in the study.

2.3. Collection of Data

In this study, researchers collected data through face-to-face interviews. A pilot study was conducted with 30 women to determine whether the questions in the survey were clear, understandable and applicable. As a result of the pilot application, no changes were made to the questions and they were included in the sample. The time it took for participants to answer the questions was approximately 15 minutes.

2.4. Data Collection Tools

In the study, an Introductory Information Form (IIF), which was prepared by making use of the literature suitable for the purpose of the study, the Birth Satisfaction Scale Revised Form (BSS-R), the Perceived Stress Scale (PSS) and the Edinburgh Postpartum Depression Scale (EPDS) were used as data collection tools.

2.4.1. IIF: It consists of a total of 17 questions, including questions about socio-demographic and obstetric information.

2.4.2. BSS-R

It is the version developed by Hollins Martin and Fleming (2011) and revised (2014). Gokmen et al. (2018) and its validity and reliability were determined by adapting it to Turkish culture. This scale has 10 items, 3 sub-dimensions and 5-point Likert answers. The names of the subscales of DMS-R are "Care Quality (CQ)", "Stress During Childbirth (SDC)" and "Personal Characteristics of Women (PCW)". The scale is scored between 0-40. A high score from the scale is interpreted as high birth satisfaction. In adapting the scale to Turkish, the Cronbach alpha coefficient was stated as 0.72 (Hollins-Martin and Martin, 2014; Gökmen, et al., 2018). In this study, 0.76 was obtained.

2.4.3. PSS

It was developed by Cohen et al. (1983). Baltaş et al. (1998) and Eskin et al. (2013) Adapted into Turkish. The purpose of developing the scale is to determine how stressful an individual perceives certain situations in his or her life. This scale is a 5-point Likert type and has 10 items. The possible score is 0-56. There are six positively worded and four negatively worded items. A high score from the scale means that the perception of stress is high. Cronbach's alpha value was reported as 0.83 (Cohen et al., 1983; Eskin, 1993). This study, 0.85 was determined.

2.4.4. EPDS

This scale was developed by Cox et al. It was adapted into Turkish by Engindeniz and others. It is used to determine the risk of PPD and measure its level and severity. The cut-off score of the four-point Likerte scale, which has 10 items, is 12/13. The score above the cut-off point indicates the risk group. Cronbach's alpha value was reported as 0.79 (Cox et al., 1987; Engindeniz et al., 1997). The authors achieved 0.77.

2.5. Statistical Analysis

IBM SPSS 26 program was used for data analysis. Distribution is given according to the socio-demographic and obstetric characteristics of the participants. Scale scores were obtained and descriptive statistics were made. Pearson correlation was used in the relationship between the overall scores of the scale, and stated that in the interpretation of the correlation coefficient, if it is above 0.70, it is at a high level, if it is between 0.40-0.70, it is at a medium level, and if it is below 0.40, it is a low level. In addition, the hierarchical regression model (enter) method was used to examine the effects of socio-demographic, obstetric characteristics, women's birth satisfaction and perceived stress levels on PPD levels. The p significance value was accepted as 0.05.

3. Results

446 women participated in the research. The average age of women was 28.48±57.14 (min-max: 19-44). According to women's socio-demographic characteristics; It was

determined that the highest percentage of people were secondary school graduates (35.4%), not working (56.5%), living in the province (54%) and their income was less than their expenses (61%) (Table 1).

According to the obstetric characteristics of women; the highest rate was multipara (67.7%), vaginal birth (54%), the last pregnancy was planned (59.4%), no problems during pregnancy (94.2%), psychiatric disease (94.4%), chronic disease (95.3%) and babies. It was determined that there was no disease (94.2%). 53.6% of the women stated that they were not satisfied with the pregnancy follow-up, 80.5% stated that they were not accompanied by a relative during the birth, and 55.8% stated that they received adequate midwife support during the birth (Table 2).

Table 1. Distribution of women according to sociodemographic variables

Variables	Group	n (%)
Education level	Primary education and below	235(52.6)
	Secondary education and above	211(47.4)
Working status	Yes	194(43.5)
	No	252(56.5)
Place of residence	City	241(54)
	County	146(32.7)
	Village	59(13.2)
Income level	Income is less than expenses	272(61)
	Income equals expenses	124(27.8)
	Income exceeds expenses	50(11.2)

Table 2. Distribution of women according to obstetric characteristics

Variables	Group	n (%)
Number of births	Primiparous	144(32.3)
	Multiparous	302(67.7)
Whether the last pregnancy was planned	Yes	265(59.4)
	No	181(40.6)
Having problems during pregnancy	Yes	26(5.8)
	No	420(94.2)
History of psychiatric illness	Yes	25(5.6)
	No	421(94.4)
History of chronic disease	Yes	21(4.7)
	No	425(95.3)
History of illness in the baby	Yes	26(5.8)
	No	420(94.2)
Desired form of birth at the beginning of pregnancy	Vaginal birth	241(54)
	Cesarean section	140(31.4)
	No preference	65(14.6)
Satisfaction with care during pregnancy follow-up	Yes	207(46.4)
	No	239(53.6)
Preferred relative's accompaniment at birth	Yes	87(19.5)
	No	359(80.5)
Receiving adequate midwife support during birth	Yes	249(55.8)
	No	197(44.2)

Descriptive statistics for each of the scale scores are given in the table, and all scores are obtained by summing the items. The total birth satisfaction score average was 22.64±10.08, and the lowest average in the sub-dimensions was found to be the personality characteristics of the woman (X=4.89±1.92) and the highest was the time in labor (X=9.12±4.04). PSS total score average was obtained as 14.63±6.77, and it can be said that there is a moderate level of stress. The mean score of the PSA positive factor subscale was 8.95±4.11, and the mean score of the negative factor subscale was 5.68±2.76. The mean EPDS total score was 8.85±4.11. Since the EPDS cut-off score average is 13, it can be said that the group is not in the risk group. In addition, a new depression variable was obtained in the form of two categories according to the EPDS cut-off score, and it was found that there was no risk of depression for 86.1% of the women and there was a risk of depression for 13.9% (Table 3).

While there was a negative and moderate relationship

between women's birth satisfaction and PPD risk (r=-0.403), a negative but low-level significant relationship was found between perceived stress (r=-0.325). As birth satisfaction increases, PPD and perceived stress levels decrease and vice versa. Additionally, there is a positive and moderate relationship between PPD risk and perceived stress (r=0.623). As the risk of PPD increases, perceived stress levels decrease and vice versa (Table 4).

Table 3. Score averages for the scale and its sub-dimensions

	CQ	SDC	PCW	BSS-R	EPDS	Positive	Negative	PSS	EPDS	
Mean	8.63	9.12	4.89	22.64	8.85	8.95	5.68	14.63	Group	n (%)
SD.	4.24	4.04	1.92	10.08	3.78	4.11	2.76	6.77		
Min.	4	4	2	10	0	6	4	10	No	384 (86.1)
Max.	16	16	8	40	22	22	14	36	Yes	62 (13.9)

CQ= care quality, SDC= stress during childbirth, PCW= personality characteristics of women, BSS-R= birth satisfaction scale-revised, EPDS= Edinburgh postpartum depression scale, PSS= perceived stress scale.

Table 4. Correlation table

	1	2	3
1. Birth Satisfaction Scale Revised Form	1		
2. Edinburgh Postpartum Depression Scale	-.403**	1	
3. Perceived Stress Scale	-.325**	.623**	1

**P<0.01

The reference categories of socio-demographic variables, which were rearranged due to the nature of the regression analysis to be two-category, are indicated in parentheses in the table. Educational status; secondary education and above / primary education and below, employment status; yes/no, place; provincial and rural; income; income equal to expenditure & more/income less than expenditure, number of births; multiparous/primiparous, last pregnancy planned; yes/no, history of psychiatric, chronic or infant illness; no/yes, type of birth; normal/cesarean section, satisfaction with pregnancy, yes/no; being accompanied by a relative at birth; yes/no and receiving adequate midwife support at birth; All categorical variables were converted into two categories as yes/no, with the reference group (1) and the control group (0) (Table 5).

For Model 1, the effect of socio-demographic characteristics on women's PPD is significant (F=11.746, p<.01). 10.8% of the variability in PPD scores is explained by the socio-demographic characteristics of women included in model 1. Age (β=0.236, 95% CI=0.1; 0.22), education level (β=-0.19, 95% CI=-2.12;-0.76), education level (β=0.114, 95% CI=0.18; 1.57), The effect of location (β=0.098, 95% CI=0.7;1.41) variables on women's PPD risk is significant. The effect of age is positive. When there is a 1 unit increase in women's age, there is a 0.236 unit increase in their PPD scores. In addition, the PPD scores of those with secondary education and above are 0.19 units lower than those with primary education and below, the PPD scores of those who are not working are 0.114 units higher than those who are employed, and the PPD scores of those living in the province are 0.098 units higher than those living in rural areas (Table 5).

For Model 2, the effect of socio-demographic and obstetric characteristics on women's PPD risk is significant (F=16.80, p<.01). 35.9% of the variability in PPD scores is explained by the variables related to women in model 2. Compared to Model 1, the disclosure rate increased to 25.1% and this increase is significant (p <.01). Age (β=0.100, 95% CI=0.01;0.13), education level (β=-0.182, 95% CI=-1.98;-0.78), location (β=0.098, 95% CI=0.09;1.25), last pregnancy being planned (β=-0.152, 95% CI=0.53; 1.81), having problems during pregnancy (β=-0.135, 95% CI=-3.93;-0.42), history of chronic disease in the baby (β=-0.23, 95% CI = -5.27; -2.16), satisfaction with pregnancy follow-up (β = 0.105, 95% CI = 0.14; 1.45) and relative accompaniment at birth (β = 0.122, 95% CI = 0.41; 1.91). The effect is significant. The effect of age is positive. When there is a 1 unit increase in women's age, their PPD scores increase by 0.100 units. In addition, the PPD scores of those with secondary education and above are 0.182 units lower than those with primary education and below, and the PPD scores of those living in the province are 0.088 units higher than those living in rural areas. While the PPD scores of those whose last pregnancy was not planned were 0.152 units higher than those whose last pregnancy was unplanned, the PPD scores of those who had no problems with pregnancy were 0.135 units lower than those who did. While the PPD scores of those whose babies do not have a disease condition are 0.23 units lower than those whose babies do not have a disease, the PPD scores of those who are not satisfied with the care during pregnancy follow-up are 0.105 units higher, and the PPD scores of women who were not accompanied by their relatives at birth are 0.122 units higher than those who were accompanied. Among socio-demographic variables, the variable with the highest impact is education level, while among obstetric characteristics, the variable with the highest impact is the variable related to whether the last pregnancy was planned (Table 5).

Table 5. Hierarchical regression table

Model	Variable	β	%95 GA	t	P
Model 1	Age	0.236	0.1;0.22	5.13	.000
	Education (Secondary education and above)	-0.19	-2.12;-0.76	-4.139	.000
	Working status (No)	0.114	0.18;1.57	2.464	0.014
	Living place (Province)	0.098	0.07;1.41	2.175	0.03
	Income (Income equal & more than expenses)	-0.074	-1.28;0.13	-1.601	0.11
	Model statistics		F=11.746** R2=0.108		
Model 2	Age	0.1	0.01;0.13	2.231	0.026
	Education (Secondary education and above)	-0.182	-1.98;-0.78	-4.511	.000
	Working status (No)	0.058	-0.18;1.06	1.398	0.163
	Living place (Province)	0.088	0.09;1.25	2.254	0.025
	Income (Income equal & more than expenses)	0.025	-0.43;0.82	0.615	0.539
	Birth number (multipara)	0.024	-0.5;0.89	0.558	0.577
	Last pregnancy planned (No)	0.152	0.53;1.81	3.607	.000
	Problems during pregnancy (No)	-0.135	-3.93;-0.42	-2.437	0.015
	History of psychiatric illness (No)	-0.063	-2.57;0.51	-1.313	0.19
	History of chronic disease (No)	-0.018	-2.38;1.74	-0.306	0.759
	Disease status of the baby (No)	-0.23	-5.27;-2.16	-4.684	.000
	Birth type (C-section)	0.037	-0.32;0.88	0.933	0.352
	Satisfied with pregnancy follow-up care (No)	0.105	0.14;1.45	2.4	0.017
	Accompaniment by a relative at birth (No)	0.122	0.41;1.91	3.035	0.003
	Receiving adequate midwife support during birth (No)	0.086	-0.04;1.34	1.864	0.063
	Model statistics		F=16.80** R2=0.359		
Model 3	Age	0.018	-0.04;0.06	0.453	0.65
	Education (Secondary education and above)	-0.131	-1.52;-0.45	-3.63	.000
	Working status (No)	-0.015	-0.65;0.43	-0.409	0.683
	Living place (Province)	0.061	-0.05;0.96	1.79	0.074
	Income (Income equal & more than expenses)	0.052	-0.14;0.95	1.461	0.145
	Birth number (multipara)	0.018	-0.46;0.74	0.471	0.638
	Last pregnancy planned (No)	0.044	-0.23;0.9	1.169	0.243
	Problems during pregnancy (No)	-0.084	-2.88;0.17	-1.746	0.082
	History of psychiatric illness (No)	-0.038	-1.96;0.71	-0.921	0.357
	History of chronic disease (No)	-0.001	-1.8;1.75	-0.027	0.979
	Disease status of the baby (No)	-0.164	-3.99;-1.28	-3.821	.000
	Birth type (C-section)	-0.007	-0.58;0.47	-0.207	0.836
	Satisfied with pregnancy follow-up care (No)	0.115	0.3;1.43	3.003	0.003
	Accompaniment by a relative at birth (No)	0.077	0.08;1.39	2.203	0.028
	Receiving adequate midwife support during birth (No)	0.003	-0.58;0.63	0.082	0.935
	Birth satisfaction	-0.218	-0.11;-0.06	-5.943	.000
	Perceived Stress	0.396	0.17;0.27	9.206	.000
	Model statistics		F=27.800** R2=0.525		

For Model 3, the effect of socio-demographic, obstetric-related characteristics and women's birth satisfaction and perceived stress levels on PPD levels is significant (F=27.800, p<.01). 52.5% of birth satisfaction is explained by the independent variables for women in model 3. According to Model 2, the disclosure rate increased to 16.6% and this increase is significant (p <.01). Education level (β=-0.131, 95% CI=-1.52;-0.45), history of chronic disease in the baby (β=-0.164, 95% CI=-3.99;-1.28), satisfaction with care during pregnancy follow-up (β=0.115, % 95% CI=0.30;1.43), presence of relatives at birth (β=0.077, 95% CI=0.08;1.39), birth satisfaction scale scores (β=-0.218, 95% CI=-0.11;-0.06) and perceived stress. The effect of the variables (β=0.396, 95% CI=0.17;0.27) on women's PPD scores is significant. Birth satisfaction has a negative effect, while perceived stress has a positive effect. When there is a 1 unit increase in women's birth satisfaction scores, their PPD scores decrease by 0.218 units, while when there is a 1 unit increase in perceived stress scores, there is a 0.396 unit increase in PPD scores. In addition, the PPD scores of those with secondary education and above are 0.131 units lower than those with primary education and below. While the PPD scores of those whose babies do not have a disease condition are 0.164 units lower than those whose babies do not have a disease, the PPD scores

of those who are not satisfied with the care during pregnancy follow-up are 0.115 units higher, and the PPD scores of women who were not accompanied by their relatives at birth are 0.077 units higher than those who were accompanied. Among the socio-demographic variables, only the education level was found to be effective, while among the obstetric characteristics, the one with the highest impact was the disease status of the baby. Perceived stress has the greatest effect among all variables (Table 5).

4. Discussion

In this study, author examined the relationship between birth satisfaction, perceived stress, and some factors and the risk of PPD, and evaluated the impact of potential factors that may increase the risk of PPD.

Author found that birth satisfaction, perceived stress and some factors had an impact on the risk of PPD, and that birth satisfaction had a negative effect and perceived stress had a positive effect. Studies in the literature have reported that birth satisfaction is a protective factor that reduces the risk of PPD (Iwata et al., 2016). Iwata et al. (2016) observed that mothers with low satisfaction with the birth process showed 2.07 more depressive symptoms than mothers who were satisfied, and their stress levels increased as they were exposed to obstetric intervention. The birth experience is one of the factors that women may remember many years later and may affect their mental health. Changes that occur with pregnancy can create burden, anxiety, and stress for the expectant mother and cause pregnancy to result in depression (Körükcü et al., 2017). It has been stated that after a birth process in which expectations are not met, the woman feels angrier, has negative emotions and thoughts, and feels inadequate in the postpartum period. Increasing satisfaction rates with quality in birth services can lead to the formation of a "healthy mother, healthy newborn and healthy society" (Moyo and Djoda, 2020).

In the current study, the authors analyzed socio-demographic characteristics; they determined that women who are older, have lower education levels and live in cities have a higher risk of PPD. In a study conducted by Dündar, he found that the rate of PPD increases as the mother gets older, and that the higher the mother's education level, the lower the rate of PPD (Dündar 2006). Although some studies conducted with mothers in the postpartum period in our country (Vural and Akkuzu, 1999) found that there was no relationship between the education level of the mothers and depression scores, Dündar (2006) and Engindeniz (1997), similar to our study, found that as the education level of the mothers increased, the PPD score decreased (Engindeniz et al., 1997; Dündar, 2006). Additionally, similar to our study, it has been reported that the risk of PPD is higher in women living in rural areas than in women living in cities (Ege et al. 2008). It is thought that increasing age and education level positively affects mothers' adaptation and coping with the new situation in the postpartum period. As women's age and education increase, their self-expression and social sharing increase, contributing to the woman's increased effectiveness in her life and effective coping with postpartum difficulties (Bingöl and Tel, 2007). In addition, it is thought that the increased risk of PPD may be due to the stressors created by urban life.

In the study, author concluded that factors such as the health status of the pregnant woman and the baby and the support of the partner/relative during birth have an impact on the risk of PPD. Similar to our study, it is known that a difficult pregnancy due to health problems, not being accompanied by a relative during birth, and endangering the baby's life increases the risk of PPD (Ahmad et al., 2021). Every mother dreams of having a healthy baby who has completed normal growth and development. Having a baby with an anomaly may cause feelings of loss and grief for a mother expecting a healthy baby (Ruschel et al., 2014). Birth is a stressful experience. In order to have this experience in a healthy and easy way, social support such as family, spouse and relatives is very important the risk of PPD may increase in mothers who have problems during pregnancy and whose babies are not healthy, as attachment adjustment becomes difficult. During the birth process, women want to have someone from their relatives with them, as well as the support of medical personnel. These people mostly consist of family members. Having anyone (spouse, mother, sibling, female relative or an experienced female relative) with the woman during the birth process creates a positive effect and increases satisfaction (Ahmad et al., 2021).

In the current study, the authors also found that unplanned last pregnancy increased the risk of PPD. Not wanting a pregnancy suggests that the mother is not ready for the baby and the role of motherhood and may have difficulties coping with the problems that may arise. In the study conducted by Dağlı et al.. it was determined that the risk of PPD was higher in mothers who became pregnant unintentionally (Dağlı et al., 2021). As a result of many studies, depression scores were found to be higher in mothers with unplanned pregnancies (Cheng et al., 2009; Dağlı et al., 2021). A woman experiencing an unwanted pregnancy will perceive the birth process negatively and her satisfaction will decrease. Unwanted pregnancy disrupts the interaction between mother and baby after birth, and the susceptibility to PPD increases in mothers who do not accept their babies (Cheng et al., 2009).

5. Conclusion and Recommendations

In this study on the risk of PPD, it was determined that birth satisfaction had a negative effect and perceived stress had a positive effect. Among the socio-demographic characteristics; age (35 years and above), education level (primary education and below); place of residence (city), obstetric characteristics; It was concluded that pregnancy planning (unplanned),

pregnancy problems (yes), baby's health (patient), pregnancy follow-up (dissatisfaction) and birth support (not accompanied by a relative) factors are effective on the risk of PPD. Perceived stress has the greatest effect compared to all variables.

Factors causing low birth satisfaction and PPD should be investigated and improved, necessary in-service training programs should be organized, and hospital procedures and health policies should be updated in the light of scientific data. It is recommended that the current study be conducted more comprehensively with different sample groups and the results be shared. In future studies, more detailed information about the development of PPD and treatment processes can be obtained by following women who are determined to be at risk of depression.

Strengths and limitations

According to the results of the scale, women who were found to be at risk of depression were contacted again on the grounds that they were at risk of depression, and they were advised to seek support from a mental health specialist. A self-report scale was used to measure mothers' PPD status in the study, and no expert evaluation was made to diagnose depression. In addition, the cultural differences and life characteristics of the research region and the fact that it was conducted in a single hospital limit the generalization of the results to other health centers and other regions of the country.

Author Contributions

The percentage of the author contributions is presented below. The author reviewed and approved the final version of the manuscript.

	E.D.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

Conflict of Interest

The author declared that there is no conflict of interest.

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Ethical Approval/Informed Consent

Ethics committee approval was received for the research by the decision of Çukurova University Medicine Non-Interventional Clinical Research Ethics Committee (approval date: 8 April 2022, protocol code: 121/72). Permission was received from the hospital (approval date: May 16, 2022, protocol code: 96172664). The importance of the subject of the research and how it would be carried out were explained to the individuals who would participate in the research, and an Informed Voluntary Consent Form was prepared to obtain informed consent from the women during the data collection phase.

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EVALUATION OF THE EFFECTIVENESS OF DIGITAL STORYTELLING ON FERTILITY AWARENESS AMONG WOMEN: A RANDOMIZED CONTROLLED EXPERIMENTAL STUDY

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Abstract: This study aims to determine the effectiveness of the digital storytelling (DS) method to raise fertility awareness (FA). This research is a pretest-posttest randomized controlled experimental study. The population of the study consisted of women who applied to the gynecology and obstetrics outpatient clinics of state hospital in March and October 2023 for who voluntarily agreed to participate in the study. Data for the study were gathered using Personal Introduction Form and Fertility Awareness Scale. physical and cognitive awareness levels of women in the intervention and control groups were similar before FA (P>0.05), and after training was given to the experimental group, all awareness levels increased significantly in the intervention group (P<0.05). When the development in each group is taken into account, there is an increase in the post-test scores in both groups. However, when the increase amounts in each group in the intervention and control groups were examined, it was determined that all awareness levels increased more in the intervention group. The DS method for gaining FA given to the intervention group was effective.

Keywords: Fertility awareness, Digital storytelling, Woman, Midwife

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1. Introduction

In order for individuals to have fertility awareness (FA), they must have knowledge about female/male genital system anatomy/physiology, fecundability, the importance of fertility and negatively affecting lifestyle behaviors (Harper et al., 2017; Pedro et al., 2018). Among the risk factors related to fertility, lifestyle-related factors such as smoking and alcohol use, insufficient exercise, sexually transmitted diseases, advancing age, caffeine consumption, obesity and stress appear to have an important place (Delbaere et al., 2020; Pedro et al., 2022). It is noteworthy that these behaviors or situations that negatively affect reproductive potential are changeable or preventable factors (Simmons and Jennings, 2020).

FA plays a key role in improving fertility self-care, increasing the chances of conception and preventing one's fears and anxiety when faced with pregnancy-related problems (Derya, 2018; Symul et al., 2019). However, it seems that there is a lack of information about lifestyle behaviors that harm fertility in the world and in our country (Moore et al., 2022). The World Health Organization recommends planning various education and health programs to ensure FA. However, there are very few studies evaluating the effects of educational interventions on FA (Wojcieszeck and Thompson, 2013; Conceição et al., 2017; Özşahin, 2020). Although educational interventions are effective, the effectiveness of a digital storytelling intervention on FA has never been evaluated.

A new health education method to increase awareness and knowledge is digital storytelling (DS). It is defined as the idea of telling a story, often with strong emotional content, using a variety of digital multimedia such as images, sound, music, and video. Using DS, applications provide deep learning and are described in the literature as an effective educational tool (Price et al., 2015; Paliadelis and Wood, 2016; Urstad et al., 2018). DS can convey many streams of information to viewers in a short time. Apart from the transferred knowledge and skills, emotions and attitudes are also transferred (Siu, 2018). DS interventions have been effective in changing other health behaviors, such as breast self-examination, prostate cancer screening, and HIV testing. Compared to written information, DS is often more accessible in terms of language and communication and can be more cost-effective. Moreover, DS can reach a wide audience very quickly through social media (Paliadelis and Wood, 2016; Siu, 2018; Urstad et al., 2018).

Creating and disseminating FA in society is important for couples to maintain a healthy perinatal period and give birth to healthy individuals, thus increasing the health level of the society. In this context, the planned study aims to determine the effectiveness of DS given to fertile women in increasing FA.

2. Materials and Methods

2.1. Study Design

This research is a pretest-posttest randomized controlled experimental study. Made according to CONSORT guidelines (Figure 1) (Schulz et al., 2010).

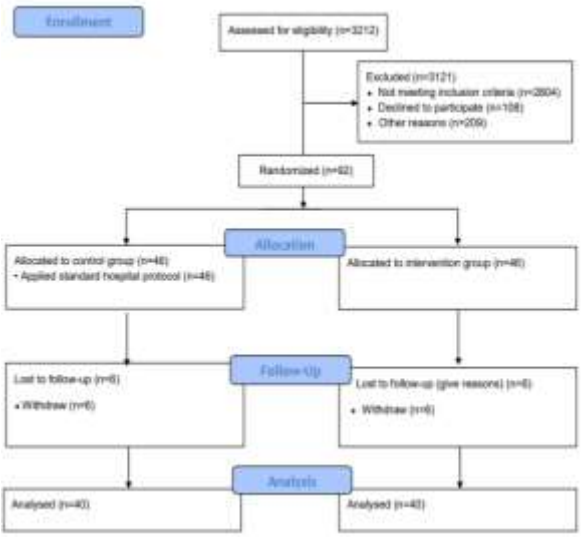

Figure 1. CONSORT flow chart

2.2. Setting and Samples

The population of the study consisted of women who applied to the gynecology and obstetrics outpatient clinics of state hospital in March and October 2023 for who voluntarily agreed to participate in the study.

The research sample consisted of a total of 80 participants, 40 experimental and 40 controls. Criteria for inclusion in the study; Being 18 years of age or older, not being pregnant, being sexually active, planning to have children, not receiving infertility diagnosis or treatment, not having been diagnosed with menopause, and not having a verbal communication problem.

G*Power application (version 3.1.9.3) was used to calculate the sample size of the study. In the literature review, it was determined that there was no study on raising fertility awareness using the digital story method, and the COHEN standard effect size was assumed to be 0.70 in determining the sample size in the study. Accordingly, the amount of Type I error is 0.05, the power of the test is 0.80 ($\alpha = 0.05$, $1-\beta = 0.80$, effect size = 0.70) and the minimal sample size is 80 according to the distribution ratio among one-to-one groups. (n=40 for each group) were found as subjects. Considering that there would be losses in cases, it was decided to recruit a total of 92 subjects (n = 46 for each group). In the study, https://stattrek.com/statistics/random-number-generator.aspx#error program was used to create intervention and control groups, and the groups were recorded by the researcher as a list (Figure 1).

2.3. Intervention Protocol

2.3.1. Intervention group (digital storytelling intervention)

A storytelling FA video intervention, approximately 10 minutes in length, guided by a situation-specific theoretical framework and storytelling/narrative communication theory (Lee et al., 2016), was developed by the researchers.

2.3.2. Control group

Routine hospital protocol was applied to the women in this group. Storytelling fertility awareness video intervention was also given to the control group after the study.

2.4. Data Collection Method and Instruments

2.4.1. Data collection tools

"Personal Information Form" and "Fertility Awareness Scale", which determine the socio-demographic characteristics of women, were used to collect data.

2.4.2. Personal introduction form (PIF)

It aims to determine the socio-demographic characteristics of women.

2.4.3. Fertility awareness scale (FAS)

This scale is a Likert-type scale consisting of 19 items and two dimensions. The lowest score that can be obtained in the total score of FAS is 19 and the highest score is 95 (Özşahin and Derya, 2022).

2.5. Data collection

In the research, the consent form was taken from women who volunteered to participate, in line with the consent of the participants at the beginning of the survey form. Data were collected face to face. Filling out the form: PIF and FAS were filled in before watching the video, and FAS was filled in again after watching the video. The data was filled in approximately 10 minutes. In practice, the FAS scale was applied separately to women in the experimental and control groups, and the results between the groups were evaluated by a statistician who was blind to the study and masked.

2.6. Evaluation of data

Independent groups' t test method was used to compare fertility awareness and its sub-dimensions between groups according to the experimental and control groups, and dependent groups t test method was used for intra-group comparison. Eta-squared effect size was calculated for the significant differences in the independent and dependent groups' t test methods. Chi-square analysis method was used for the relationship between demographic categorical variables and groups. All statistical analyzes were examined at the $P<0.05$ significance level.

3. Results

There is no significant difference as a result of the chi-square analysis between the demographic variables of women in the intervention and control groups ($P>0.05$). In other words, the rates of education level, employment status, place of residence and income level of patients in the intervention and control groups are similar. The ages of the patients in the intervention and control groups were compared using the independent groups t test method, and there was no significant difference between the women's groups according to their ages ($P>0.05$).

The average age of patients in the intervention and control groups is similar (Table 1). There is no significant difference between the FAS total and subscale pretest scores of women in the intervention and control groups (P>0.05) and the pretest score averages are similar (Table 2). A significant difference was obtained between the FAS total and subscale posttest scores of women in the intervention and control groups (P<0.05). The FAS total posttest mean scores and the physical and cognitive subscale posttest mean scores of women in the intervention group are higher than those of women in the control group (Table 2). There is a significant difference between the pretest and posttest scores of women in the intervention group on the FAS total (t=-34.269, P>0.05), physical (t=5.405, P>0.05) and cognitive (t=-5.063, P>0.05) subscales. In the intervention group, women's FAS total posttest mean score and physical and cognitive subscale posttest score mean were higher than the pretest (Table 2). There was a significant difference between the pretest and posttest scores of the FAS total (t=-46.996, P>0.05), physical (t=-3.365, P>0.05) and cognitive (t=-2.308, P>0.05) subscales of women in the control group. There is difference. The FAS total posttest mean score and the physical and cognitive subscale posttest score mean of the women in the control group are higher than the pretest (Table 2).

Table 1. Comparison of socio-demographic characteristics of women

Variable	Group	Intervention (N=40)	Control (N=40)	Statistics	p
		f(%)	f(%)		
Education	Primary education and below	13(32.5)	15(37.5)	$\chi2$=0.22	0.639
	Secondary education and above	27(67.5)	25(62.5)		
Working status	Yes	31(77.5)	28(70)	$\chi2$=0.581	0.446
	No	9(22.5)	12(30)		
Place of residence	Province	23(57.5)	20(50)	$\chi2$=0.916	0.633
	District	11(27.5)	15(37.5)		
	Village	6(15)	5(12.5)		
Income level	Income is less than expenses	19(47.5)	17(42.5)	$\chi2$=0.229	0.892
	Income equals expenses	16(40)	18(45)		
	Income exceeds expenses	5(12.5)	5(12.5)		
Age (Mean±sd)		28.73±4	27.38±4.87	t=1.356	0.179

χ^2= Chi-square test statistic; t= independent groups t test statistics

Table 2. Comparison of women's fertility awareness scale total and sub-dimension scores within and between groups

Scale Scores	Intervention (N=40)	Control (N=40)	[a]Test request & p value	Eta squared
	Mean±sd	Mean±sd		
Physical pretest	30.63±3.39	31.58±3.03	t=-1.321 p=0.19	
Physical posttest	35.35±4.08	32.03±3.32	t=4 p=.000	0.17
[b]Test request & p value	t=-5.405 p=.000	t=-3.365 p=0.002		
Eta kare	0.43	0.23		
Cognitive pretest	23.4±2.28	22.63±2.36	t=1.492 p=0.14	
Cognitive posttest	28.2±5.2	23.35±3.14	t=5.045 p=.000	0.25
[b]Test request & p value	t=-5.063 p=.000	t=-2.308 p=0.026		
Eta kare	0.40	0.12		
FAS total pretest	54.03±3.63	54.2±4.18	t=-0.2 p=0.842	
FAS total posttest	82.23±5.68	77.55±6.63	t=3.387 p=0.001	0.13
[b]Test request & p value	t=-34.269 p=.000	t=-46.996 p=.000		
Eta squared	0.97	0.98		

a= independent groups t test statistical value; b= dependent groups t test statistical value, FAS= fertility awareness scale

4. Discussion

In this study, the effectiveness of the DS method for gaining FA in women was evaluated. The DS method was effective in the intervention group. To the best of the author's knowledge, this study is the first randomized controlled study investigating the effect of the DS method on FA. Since there is no study on the effect of the DS method on FA, the study findings are discussed within the framework of the results of other studies.

In this study, the physical and cognitive awareness levels of women in the intervention and control groups were similar before FA, and after training was given to the experimental group, all awareness levels increased significantly in the intervention group. When the development in each group is taken into account, there is an increase in the post-test scores in both groups. However, when the increase amounts in each group in the intervention and control groups were examined, it

was determined that all awareness levels increased more in the intervention group. Willis et al. (2014) applied DS therapy to 12 HIV-positive adolescents, and in the therapies, the adolescents were asked to express their feelings and create their own stories. As a result of the study, adolescents declared that it increased their self-confidence and belief. Laing et al. (2017a), 16 adolescents were asked to prepare training and digital stories about the DS method. In the interview conducted after the completion of the stories, the adolescents stated that digital stories were a way to understand others' cancer experiences and to tell their own experiences. As a result of the study, the DS method was shown as a promising method to reduce psychosocial negativities in the treatment and care of adolescent oncology patients, and it was recommended to include digital stories in clinical practice and develop follow-up programs (Laing et al., 2017b). There are many experimental studies in the literature showing that the DS method facilitates learning, provides in-depth understanding and remembering, and improves critical thinking-research and information analysis skills (Gubrium et al., 2015; Conceição et al., 2017). Digital stories are reflective, creative and value-laden, revealing important things about the human condition (Siu, 2018). DS is one of the methods increasingly used in health promotion efforts (Price et al., 2015). Patients in the digital narrative have the potential to be portrayed at various stages of health and disease throughout their lives (Ogston-Tuck et al., 2016).

5. Conclusion and Recommendations

The DS method for gaining FA given to the intervention group was effective. Developing FA is a current issue that will improve maternal and child health, and therefore public health. It is recommended to conduct studies that more comprehensively compare the use of the DS method in the training of midwives and its effect with other intervention formats.

Author Contributions

The percentage of the author contributions is presented below. The author reviewed and approved the final version of the manuscript.

	E.D.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

Conflict of Interest

The author declared that there is no conflict of interest.

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Ethical Approval/Informed Consent

Ethics committee approval was received for the research by the decision of Çukurova University Medicine Non-Interventional Clinical Research Ethics Committee (approval date: dated 4 Feb 2023, protocol code: 130/76). Permission was received from the hospital. Written consent was obtained from the participating in the research by means of an Informed Voluntary Consent Form. All procedures were in accordance with the 1964 Helsinki Declaration of Human Rights and its subsequent amendments or comparable ethical standards. The subjects were free to discontinue their participation at any stage.

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İNAKTİF SARS-COV-2 AŞISI UYGULANAN SAĞLIK ÇALIŞANLARINDA ANTI-SPIKE S1 RBD IGG DEĞERLERİNİN BELİRLENMESİ

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Özet: COVID-19 pandemisi sırasında hastalık ve komplikasyonlarıyla mücadele amacıyla çeşitli aşılar geliştirilmiştir. Çalışmamızda iki doz Coronavac (Sinovac) aşısı uygulanmış sağlık çalışanlarında COVID-19 antikor titrelerinin belirlenmesi amaçlanmıştır. Mart- Eylül 2021 tarihleri arasında Mersin Üniversitesi Hastanesi'nde gönüllü sağlık çalışanlarından iki doz Coronavac aşısı uygulanan 186 kişiden alınan kan örneklerinden Access SARS-CoV-2 IgG testi (Beckman) uygulandı. Antikor titreleri ELISA temelli test ile tespit edildi. Çalışmaya dahil edilen 186 katılımcının %47,8'i (n=89) erkek, %52,2'si (n=97) kadındır. Yaş ortalaması 42,3±8,7 (23-60) 'tür. 40 yaş ve altı katılımcılar ile 40 yaş üzeri katılımcıların pozitiflik oranları arasında anlamlı farklılık tespit edildi (<=40; %68,1, >40; %43,6, P=0,001). En fazla pozitif antikor titresi olan grup 21-30 yaş grubu olduğu görüldü. Yaş artışı ile birlikte antikor düzeyleri anlamlı şekilde azaldığı belirlendi (r=-0.203, P=0,001*).* Ek hastalığı olan 22 katılımcı vardır. Ek hastalığı olanlarda, sağlıklı olanlara göre antikor titresi açısından anlamlı farklılık gözlenmedi. Çalışmamız kısa süreli bir proje olduğu için az sayıda katılımcı ile yapılmıştır. Daha geniş kapsamlı yapılacak çalışmalara ihtiyaç vardır.

Anahtar kelimeler: COVID-19, İnaktif aşı, Antikor

Determination of Anti-Spike S1 RBD IgG Values in Healthcare Workers with Inactivated SARS-CoV-2 Vaccine

Abstract: Various vaccines have been developed to combat the disease and its complications during the COVID-19 pandemic. Our study aimed to determine COVID-19 antibody titers in healthcare workers who received two doses of Coronavac (Sinovac) vaccine. Access SARS-CoV-2 IgG test (Beckman) was performed on blood samples taken from 186 volunteer healthcare workers who received two doses of Coronavac vaccine at Mersin University Hospital between March and September 2021. Antibody titers were determined by ELISA-based testing. Of the 186 participants included in the study, 47.8% (n = 89) were male and 52.2% (n = 97) were female. The average age is 42.3±8.7 (23-60). A significant difference was detected between the positivity rates of participants aged 40 and under and participants over 40 years of age (<=40; 68.1%, >40; 43.6%, P=0.001). It was observed that the group with the highest positive antibody titer was the 21-30 age groups. It was determined that antibody levels decreased significantly with increasing age (r=-0.203, P=0.005). There were 22 participants with comorbidities. No significant difference was observed in terms of antibody titer in patients with comorbidities compared to healthy individuals. Since our study was a short-term project, it was conducted with a small number of participants. More comprehensive studies are needed.

Keywords: COVID-19, Inactivated vaccine, Antibody

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1. Giriş

Şiddetli akut solunum sendromu koronavirüs 2 (SARS-CoV-2)' nin neden olduğu 2019 koronavirüs hastalığı (COVID-19) dünya çapında 700 milyondan fazla insana bulaştığı bilinmektedir. COVID-19 yaklaşık 7 milyon insanın ölümüne yol açtı (WHO, 2023; Siracusano ve ark., 2020). Aynı zamanda bu süreçte birçok SARS-CoV-2 varyantı meydana geldi ve 2020 yılı sonlarında ilk önce İngiltere'de tanımlanan alfa varyantı ve daha sonra Hindistan'da tanımlanan delta varyantı daha yüksek bulaş özelliği ile baskın varyant tipi olarak etkisini sürdürmektedir (Firestone ve ark., 2021). SARS-CoV-2 Coronaviridae familyasındaki betakoronavirüs cinsine ait pozitif polariteli zarf içeren RNA virüsüdür (Siracusano ve ark., 2020). SARS-CoV-2 yapısındaki spike (S), nükleokapsit (N), zarf (E) ve membran (M) proteinleri immün sistemde görevli B ve T lenfositlerin oluşturduğu immün yanıtta antijenik özellik göstermektedir. Genel

olarak S proteini bu bağışıklık yanıtında çok önemli bir rol oynamaktadır. COVID-19'a karşı aşı geliştirilmesinde de bu proteinler hedef alınarak çalışılmalar yürütülmektedir (Dong ve ark., 2020). Yeterli düzeyde bağışıklık için B lenfositlerin oluşturduğu spesifik antikor yanıtı büyük öneme sahiptir. Bu antikor yanıtının oluşumunda aşılar bir çözüm olarak karşımıza çıkmaktadır (Harrison ve Wu, 2020). Yapılan çalışmalarda SARS-CoV-2 seropozitif iyileşmiş bireylerin yeniden enfeksiyona karşı %89 korumaya sahip olduğu ve aşı etkinliklerinin %50 ila %95 arasında olduğu rapor edilmiştir (Lumley ve ark., 2021). Salgının önlenmesine yönelik farklı türde aşılar geliştirilmiş olup, hali hazırda Türkiye'de kullanılan inaktif (CoronaVac; Sinovac) ve mRNA (BNT162b2; Pfizer–BioNTech) aşıları mevcuttur (WHO, 2020).

CoronaVac aşısı Türkiye'nin de aralarında bulunduğu birkaç ülkede acil kullanım onayı almıştır. Türkiye'de ilk olarak 14 Ocak 2021 tarihinde sağlık çalışanlarına uygulanmaya başlanmıştır ve faz 3 çalışmalarında ikinci doz aşıdan 14 gün sonra aşının etkinliği %83,5 olarak bildirilmiştir (Tanriover ve ark., 2021). Ancak aşının etkinliğinin ne kadar sürdüğü ilgili çalışmalar devam etmektedir.

Bu çalışmada Mersin Üniversitesi Tıp Fakültesi Hastanesinde çalışmakta olan sağlık çalışanlarında CoronaVac aşısı sonrası anti-spike S1 RBD (Reseptör Bağlanma Bölgesi) IgG değerlerinin belirlenmesi, yaş ve cinsiyete göre değişkenliklerinin ve ek hastalıklarla ilişkisinin değerlendirilmesi amaçlandı.

2. Materyal ve Yöntem

2.1. Çalışmanın Tasarımı

Tek merkezde ve üçüncü basamak bir hastanede sağlık çalışanları ile uygulanan prospektif klinik bir çalışmadır.

2.2. Çalışma Grubu

Bu çalışmaya Mersin Üniversitesi Tıp Fakültesi Hastanesinde görevli olan 186 gönüllü sağlık çalışanı katıldı. 15 Mart 2021 ve 15 Eylül 2021 tarihleri arasında öncesinde COVID-19 hastalığı geçirme öyküsü olmayan ve iki doz CoronaVac aşısı uygulanan 18-65 yaş arası sağlık çalışanları bu çalışmaya dahil edildi. İmmünsupresif tedavi almak, tek doz aşılanmış ya da hiç aşılanmamış olmak dışlama kriteri olarak belirlendi.

2.3. Örneklerin Toplanması

Mersin Üniversitesi Tıp Fakültesi Hastanesi'nde, bu araştırmaya gönüllü olan sağlık çalışanlarından, ikinci doz CoronaVac aşısını yaptırdıktan 30±2 gün sonra kan örnekleri alındı. Kan alma işlemi hastanenin kan alma biriminde yapıldı ve ardından çalışma için uygun saklama koşullarında laboratuvara ulaştırıldı. Örnekler bekletilmeden 4000 rpm devirde 10 dakika santrifüj edilerek serum elde edildi ve serumlar anti-spike S1 IgG tespit aşamasına kadar -20 ˚C'de derin dondurucuda saklandı.

2.4. Anti-Spike S1 IgG tespiti

SARS-CoV-2 anti-spike S1 RBD IgG tespiti için paramanyetik partiküllü kemilüminesan bir immünoanaliz yöntemi olan Access SARS-CoV-2 IgG (Beckmann Coulter, ABD) testi kullanıldı. Access SARS-CoV-2 IgG testi S1 proteininin reseptör bağlanma alanına (RBD) özgü oluşan antikor yanıtı tespit eder. Üretici firma tarafından bu testin klinik duyarlılığı %100 (>18 gün), özgüllüğü %99,8 olarak bildirilmiştir.

2.5. Test Prensibi

Access SARS-CoV-2 IgG testi, iki adımlı bir enzim immünoanalizidir. S1 proteininin reseptör bağlanma alanı (RBD) için spesifik rekombinant SARS-CoV-2 proteini ile kaplanmış paramanyetik partikülleri ve tamponu içeren reaksiyon küvetine bir örnek eklenir. Reaksiyon küvetinde inkübasyondan sonra katı faza bağlı materyaller manyetik alanda tutulur, bağlı olmayan materyaller yıkanıp temizlenir. Monoklonal anti-insan IgG alkalin fosfataz konjugatı eklenir ve konjugat, partiküllerde yakalanan IgG antikorlarına bağlanır. İkinci bir ayırma ve yıkama adımı, bağlanmamış konjugatı giderir. Kemilüminesan substrat küvete eklenir ve reaksiyon ile üretilen ışık bir lüminometre ile ölçülür. Işık üretimi, cihazın kalibrasyonu sırasında tanımlanan kesim değeriyle karşılaştırılır.

2.6. Sonuçların Yorumu ve Raporlama

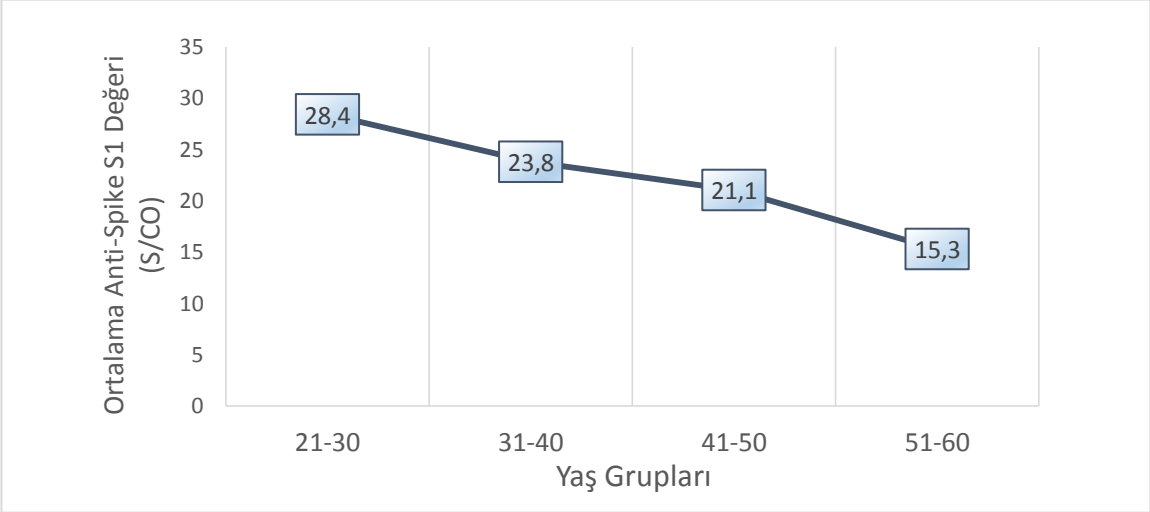
Test sonuçları sistem yazılımı tarafından otomatik olarak belirlendi. Elde edilen sonuçlar üretici firma önerileri doğrultusunda değerlendirildi. Access SARS-CoV-2 IgG testine ait ≤0,8 S/CO olan sonuçlar negatif, >0,8 ile <1,0 S/CO olan sonuçlar belirsiz, ≥1,0 S/CO olan sonuçlar pozitif olarak yorumlandı.

2.7. İstatistiksel Analiz

Elde edilen tüm veriler SPSS 20.0 paket programına (IBM, Armonk, NY, ABD) girilerek analiz edildi. Normallik testi için test sonuçlarına Log dönüşümü uygulandı. Gruplar arası karşılatırmada ki-kare testi, test sonuç değerlerinin analizinde bağımsız değişken t-testi ve ANOVA testi kullanıldı. P değeri <0,05 anlamlı kabul edildi.

3. Bulgular

Bu çalışmaya dahil edilen 186 katılımcıdan 97 (%52,2)'si kadın, 89 (%47,8)'u erkek sağlık çalışanıydı. Katılımcıların yaş ortalaması 42,3±8,7 (Min/Max:23/60) olarak tespit edildi. Toplamda SARS-CoV-2 anti-spike S1 RBD IgG serokonversiyon oranı %97,3 (181/186) bulundu. Cinsiyete göre serokonversiyon oranları kadınlarda %96,9, erkeklerde %97,8 idi. Kadın ve erkeklerde anti-spike S1 RBD IgG değerlerinin ortalaması sırasıyla 21,2±33,9 S/CO ve 22,5±53,7 S/CO olarak hesaplandı (P>0,05). Cinsiyete göre anti-spike S1 IgG RBD sonuçlarında anlamlı bir farklılığa rastlanmadı. Katılımcılar yaş gruplarına ayrılıp incelendiğinde yaş artışı ile antikor değerlerinin azaldığı gözlendi (Şekil 1).



Şekil 1. Yaş gruplarına göre Anti-Spike S1 değerleri.

Kırk yaş ve altı sağlık çalışanlarında tamamı (n=69) seropozitif bulunurken, 40 yaşın üzerindekilerin %95,7 (112/117)'si seropozitif olarak bulundu. Ortalama antikor değerleri 40 yaş ve altı kesimde (25,8±39,2 S/CO), 40 yaşın üzerindekilere göre (19,5±47,1 S/CO) daha yüksek tespit edildi ve istatistiksel olarak anlamlı bulundu (P=0,002) (Tablo 1). Kırk yaş ve altı grupta cinsiyete göre antikor değerlerinde farklılığa baktığımızda kadınlarda antikor değerleri (n=40; 31,0±45,8 S/CO) erkeklerin antikor değerleri (n=29; 18,5±26,8 S/CO) göre daha yüksek bulundu. Kırk yaş üstü katılımcılarda ise tam tersi bir durum geçerliydi; kadınlardaki antikor değerleri (n=57, 14,3±19,8 S/CO) erkeklerin antikor değerlerine (n=60, 24,4±62,8 S/CO) göre daha düşük tespit edildi. Ancak istatistiksel olarak bu iki durum için de anlamlılık tespit edilmedi (P=0,091, P=0,534).

Katılımcıların ek hastalıkları incelendiğinde toplamda 22 sağlık çalışanı ek hastalığı olduğunu belirtti. Bu hastalıklar tiroid hastalıkları (n=7), geçirilmiş malignensi öyküsü (n=5), hipertansiyon (n=4), diyabet (n=3), inflamatuar barsak hastalığı (n=1), alerjik rinit (n=1) ve fibromyalji (n=1) şeklindeydi. Ek hastalık belirten katılımcılarla (Tablo 2) sağlıklıları karşılaştırdığımızda serokonversiyon oranları ve antikor değerlerinde anlamlı bir farklılık gözlenmedi (P=0,273, P=0,276).

Tablo 1. CoronaVac aşısı sonrası yaşa ve cinsiyete göre antikor yanıtı

	SARS-CoV-2 Anti-Spike S1 RBD IgG			
	Pozitiflik % (n/Toplam)	P Değeri	Ort. Değerler (S/CO) ±SD	P Değeri
Cinsiyet				
Kadın	96,9 (94/97)	>0,05[a]	21,2 ±33,9	>0,05[b]
Erkek	97,8 (87/89)		22,5 ±53,7	
Yaş Grubu				
≤40	100,0 (69/69)	>0,05[a]	25,8 ±39,2	0,002[b]
>40	95,7 (112/117)		19,5 ±47,1	
Toplam	97,3 (181/186)		21,8 ±44,4	

[a]Ki-kare testi, [b]Bağımsız değişken t-testi

Tablo 2. Katılımcıların ek hastalıklarına göre dağılımları

Ek Hastalık	IgG Sonucu	Katılımcı Sayısı (n)	%
Alerjik Rinit	Pozitif	1	100
Diyabet	Negatif	1	33,3
	Pozitif	2	66,7
Enteropati	Pozitif	1	100
Hipotroidi	Negatif	1	33,3
	Pozitif	2	66,7
Malignite (Kemoterapotik)	Negatif	2	40,0
	Pozitif	3	60,0
Fibromiyalji (Pregabalin)	Pozitif	1	100
Hipertansiyon	Negatif	3	75,0
	Pozitif	1	25,0
Hipertiroidi	Negatif	1	25,0
	Pozitif	3	75,0

4. Tartışma

Pandeminin başlangıcından bu yana aşı çalışmaları devam ederken, birçok farklı teknik kullanılarak üretilen aşılardan hangisinin bu virüse ve oluşan varyantlarına daha etkili olduğu tartışması gündemden düşmeyen bir konudur (Souza ve ark., 2021). Aşının etkinliğinin tespiti için SARS-CoV-2 spesifik antikor testlerinin kullanımı konusunda da tartışmalar devam etmektedir. Yapılan bazı in vitro çalışmalarda Nötralizan antikor (NAb) – SARS-CoV-2 Spike RBD IgG korelasyonunun yüksek olduğu gösterilmiştir (Dogan ve ark., 2021). Bu çalışmada kullandığımız test ile sağlık çalışanlarının iki doz CoronaVac aşılanması sonrası SARS-CoV-2'ye karşı oluşturdukları immün yanıt analiz edildi. Ülkemizde ve dünyada iki doz CoronaVac aşılama sonrası serokonversiyon oranlarının %75,4 ila %100 arasında değiştiği çalışmalara rastlanmıştır (Binay ve ark., 2021; Bayram ve ark., 2021; Zee ve ark., 2021; Davarcı ve ark., 2021; Zhang ve ark., 2021; Bichara ve ark., 2021). Bu çalışmada tespit ettiğimiz %97,3 serokonversiyon oranı ülkemizde yapılmış çalışmalar ile benzer niteliktedir.

Sağlık çalışanlarında, önerilen programa göre 21 gün arayla iki doz Pfizer-BioNTech BNT162b2 aşılamanın ardından anti-spike IgG düzeylerinin bakıldığı bir kohort çalışmada kadınların antikor düzeyleri erkeklere göre daha yüksek tespit edilmiştir; ancak istatistiksel olarak anlamlı bulunmamıştır (Anastassopoulou ve ark., 2022). Ülkemizde sağlık çalışanlarında yapılmış bir çalışmada inaktif Coronavac aşılaması ile ilk doz aşılamadan sonra antikor pozitifliği yüzdesi erkeklerde %51,1 ve kadınlarda %42,0 olarak bulunmuştur. İkinci doz aşılamadan sonra antikor pozitifliği yüzdesi erkeklerde %99,5 ve kadınlarda %99,2 olarak bulunmuştur. CoronaVac aşısının etkinlik oranı hem 40 yaş altı hem de 41 yaş üstü tüm katılımcılarda yüzde 99,4 olarak belirtilmiştir (Dinc ve ark, 2022). Çalışmamızda erkek katılımcıların pozitiflik yüzdesi (%97,8) kadın katılımcılara (%96,9) göre daha fazladır. Ancak istatistiksel olarak cinsiyete göre anlamlı bir farka rastlanmamıştır. Aşı etkinlik oranı literatürdeki diğer çalışmalara paralel şekilde yüksek bulunmuştur.

Çalışmalarda yaş grupları ile antikor oluşturma düzeyleri arasında anlamlı ilişki saptanmıştır. Yaş ilerledikçe antikor düzeylerinin azaldığı belirtilmektedir (Anastassopoulou ve ark., 2022). Pediatrik ve erişkin hastalarla yapılan kesitsel bir çalışmada benzer seroprevalansa rağmen IgG düzeylerinin farklı yaş gruplarında farklılık gösterdiği vurgulanmıştır. SARS-CoV-2 IgG seviyesi, pediatrik popülasyonda yaş ile negatif bir korelasyon ($r= -0,45$, $P<0,001$) ve yetişkinlerde yaş ile pozitif bir korelasyon göstermiştir ($r= 0,24$, $P<0,001$) (Yang ve ark., 2021). Çalışmamızda sadece erişkin yaş grubunda antikor düzeylerine bakılmış ve yaş grupları ile değerlendirildiğinde en yüksek değerler 21-30 yaş aralığında olduğu görülmüştür ($P<0,05$).

SARS-CoV-2'ye karşı aşılamanın, COVID-19'dan kaynaklanan enfeksiyonları, hastaneye yatışları ve ölümleri önlemede başarılı olduğu kanıtlanmıştır. Ancak, pandemi süreci başlarında pandemi ile savaşa öncelik verildiği için, immünsupresif ilaçlarla tedavi edilen veya ek hastalığı olan hastalarda SARS-CoV-2 aşılarının etkinliği aşılar yaygın olarak kullanıma girdikten sonra yapılabilmiştir. İnflamatuar barsak hastalığı tanılı bireylerde COVID-19 aşılarının immünojenitesi, immünsüpresif ilaç maruziyetine göre değiştiğini belirten çalışmalar mevcuttur (Alexander ve ark., 2022). Aşılama oranlarının yüksek olduğu popülasyonlarda SARS-CoV-2 enfeksiyonuna bağlı ölümlerin azaldığı bilinmektedir. Ancak, ileri yaş (>65 yaş) veya komorbitesi olan gruplarda aşı etkinliğinin hızla azaldığı ve buna bağlı olarak şiddetli SARS-CoV-2 enfeksiyonu açısından riskin devam ettiği vurgulanmıştır (Adab ve ark., 2022). Çalışmamızda ek hastalığı olduğu tespit edilen gönüllülerin antikor düzeyleri diğer gönüllüler ile karşılaştırıldığında anlamlı bir farklılık tespit edilmemiştir.

5. Sonuç

Aşılanmış gönüllülerde antikor titrelerinin pozitif çıkması aşılamanın hastalığa karşı koruyuculuğunu göstermiştir. Pandemi sürecinde yeni geliştirilen aşıların önümüzdeki süreçte tekrarlayan salgın periyotlarında etkili olabileceğini göstermesi açısından verilerimizin literatüre katkı sağlayacağını düşünmekteyiz.

Çalışmanın Kısıtlılıkları

Çalışmamız kısa süreli bir periyotta gerçekleşmiş olup bütçe kısıtlılıkları sebebiyle az sayıda gönüllü ile yapılmıştır. Nötralizasyon testlerinin yapılamaması sebebiyle aşı sonrası oluşan antikorların etkinliği tespit edilememiştir.

Katkı Oranı Beyanı

Yazar(lar)ın katkı yüzdesi aşağıda verilmiştir. Tüm yazarlar makaleyi incelemiş ve onaylamıştır.

	K.B	T.B.	H.K.	H.G.	S.T.Ü.	G.A.
K	30	10	10	10	10	30
T	20	20	10	10	10	30
Y	20	20	20	10	10	20
VTI	40	20	20			20
VAY	20	20	20	10	10	20
KT	20	20	20	10	10	20
YZ	20	20	20	10	10	20
KI	20	30	20			30
GR	20	30	20	10	10	10
PY	20	20	20	10	10	20
FA						100

K= kavram, T= tasarım, Y= yönetim, VTI= veri toplama ve/veya işleme, VAY= veri analizi ve/veya yorumlama, KT= kaynak tarama, YZ= Yazım, KI= kritik inceleme, GR= gönderim ve revizyon, PY= proje yönetimi, FA= fon alımı.

Çatışma Beyanı

Yazarlar bu çalışmada hiçbir çıkar çatışmasının olmadığını beyan etmektedirler.

Etik Onay/Hasta Onamı

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Teşekkür ve Destek Beyanı

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Research Article
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THE EFFECT OF NECROSTATIN -1 AND ENOXAPARIN MOLECULES ON RANDOM PATTERN FLAP VIABILITY

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Abstract: Distal flap necrosis is seen more often in random pattern flaps and is an important complication that shortens the flap length. There has been much research many drugs and molecules in an effort to prevent this complication. The aim of this study was to investigate the efficacy of necrostatin-1 and enoxaparin molecules in preventing distal flap necrosis and increasing flap viability in a random pattern flap model created in rats. A total of 32 Wistar albino female rats, each weighing 300-350 gr were separated into 4 groups. All the animals underwent an operation to create a 3×9 cm caudal-based Mcfarlane flap. The treatments defined for each group were applied. Full layer tisssue samples 1×1 cm^2 were taken from all the flaps and stored until histopathological and immunohistochemical examination, the parameters of inflammation, capillary proliferation, necrosis, fibroblast proliferation and fibrosis were compared histopathologically. In the necrostatin-1 group, the inflammation, necrosis and fibrosis scores were observed to be lower and the capillary proliferation and fibroblast proliferation scores were higher. In the enoxaparin group, the fibroblast proliferation and capillary proliferation scores were higher. The receptor interacting protein kinase-1 immunohistochemical staining results showed statistically significantly less staining in the necrostatin-1 group compared to the other groups. The results of this study suggest that necrostatin molecule has important therapeutic potential in increasing flap viability in the random pattern flap model, considering the percentage of flap necrosis, and the immunohistochemical and histopathological data. The flap necrosis percentage and histochemical parameters of the enoxaparin molecule demonstrate that the effects on flap viability are limited.

Keywords: Necrostatin-1, Enoxaparin, Rat, Flap necrosis, Flap viability

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1. Introduction

Tissue loss occurring after trauma or oncology surgery is a significant medical problem which results in severe morbidity and mortality (Gottlieb and Krieger, 1994). A flap can be basically defined as a piece of tissue transferred from one region to another while protecting the vascular structure (Janis, 2014). Flaps are classified in two groups according to the vascular structure, as axial pattern or random pattern flaps. As the application of random pattern flaps is simple, they are often used in reconstructive surgery for the repair of tissue defects (McGregor and Morgan, 1973). The most significant problem encountered after flap surgery is flap necrosis and partial or full flap loss. Distal flap necrosis seen especially in random pattern flaps is a significant problem (Janis, 2014). The need to increase flap length which can be used in this type of flap without distal flap necrosis developing has led to many studies having been conducted (Taylor et al., 1992).

Necroptosis is defined as programmed cell death, similar to the morphologically necrotic cell death that is seen in ischaemic tissues (Christofferson and Yuan, 2010). This pathway is regulated by tumour necrosis factor receptor (TNFR) and Fas ligand receptor activation, independently of the caspase system. Receptor interacting protein kinase-1 (RIPK-1) and receptor interacting protein kinase-3 (RIPK-3) activity is required for TNFR activation (Degterev et al., 2005; Xie et al., 2013). In recent studies, necroptotic cells have been shown in ischaemic-reperfusion created in rat skin flap models and it has been reported that necroptosis could have a role in flap necrosis (Liu et al., 2019a). Due to this role of RIPK-1 and RIPK-3 in necroptosis, it has been considered that inhibition of these enzymes could be a good strategy

against necroptosis. Degterev defined necrostatin-1 (nec-1) specific to RIPK-1 as a compound that blocks necrotic cell death in human and rat cells (Degterev et al., 2005).

The aim of this study was to examine and compare the effects on flap viability of necrostatin-1 and enoxaparin, which is a low-molecular weight heparin (LMWH), molecules in a rat model of random pattern skin flaps as described by McFarlane.

2. Materials and Methods

Approval for the study was granted by the Animal Experiments Local Ethics Committee of Kahramanmaras Sutcu Imam University (KSU) (protocol no: 18, session no:2021/07, decision no: 01, dated: 13.10.2021). The study was supported by the Scientific Research Projects Unit of KSU Experimental Animals Centre (project no: 2021/7-4D).

2.1. Formation of the study groups

The rats were separated into 4 groups of 8 animals. A caudal-based flap operation was performed to all groups in the McFarlane flap model.

- Group 1: No treatment was applied to this group.
- Group 2: Dimetil sülfoksit (DMSO)+isotonic mixture was administered intraperitoneally as a single daily dose of 0.5 ml starting with the first dose 1 hour before the operation.
- Group 3: Enoxaparin molecule (diluted in isotonic) was administered subcutaneously as a single daily dose of 100 IU/kg starting with the first dose 30 mins before the procedure.
- Group 4: Necrostatin-1 molecule was administered intraperitoneally (dissolved in DMSO+isotonic) as a single daily dose of 1.65 mg/kg starting with the first dose 1 hour before the operation.

2.2. Study Protocol

2.2.1. Necrostatin-1 molecule preparation

Necrostatin-1 molecule was purchased from Cayman Chemical company (Item no:11658) in powder form and was prepared for use in the University – Industry – Public Works Collaboration Development Practice and Research Centre (ÜSKİM). The molecules were first dissolved in DMSO solution to be 14 mg/ml, and were then diluted with isotonic solution to be at a concentration of 1mg/ml.

2.2.2. Surgical procedure

Before the surgical procedure, all the rats were injected intraperitoneally with Ketamin (50 mg/kg) (Ketalar, Eczacıbaşı, Türkiye) and Xylazine HCl (5mg/kg) (RompunR, Bayer, Türkiye), and thus general anaesthesia was obtained. The landmarks for the area where the flap was planned on the dorsal region of each rat were marked as both scapulae and the posterior iliac notches. A caudal-based McFarlane skin flap, 3 x 9 cm in size, was drawn with a skin pen, and the flap plan was formed. The surgical area was stained with povidone iodine, then following sterile draping, the flap was raised in the loose avascular plane over the dorsal muscles including the subcutaneous panniculus carnosus muscle. Hemostasis was obtained then the incision areas were closed by primary suturing returning the flap to its place. The surgical procedure was terminated (Figure 1). Postoperatively, the rats were followed up daily for 7 days, and at the end of 7 days, all the rats were sacrificed with high-dose anaesthesia.

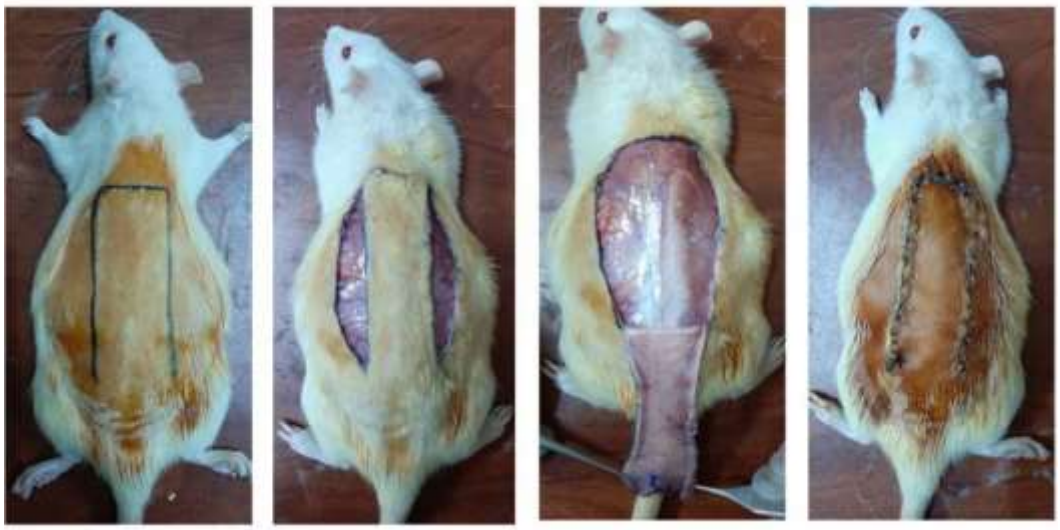

Figure 1. Surgical stages of the McFarlane skin flap.

2.3. Calculation of the Flap Necrosis Area

On postoperative day 8 after the rats were sacrificed, photographs were taken. The digital photographs obtained were uploaded to a computer, and using ImageJ 1.53 program, the total flap area and necrotic area were calculated for all the groups. The necrotic area was stated as the necrotic area percentage of the total flap area (Figure 2).

2.4. Histopathological and Immunohistochemical Evaluations

On postoperative day 8 after the rats were sacrificed, a full layer approximately 1 x 1 cm^2 tissue sample was

taken from the demarcation zone passing through the live line-necrosis line of the flap tissue. All the tissue samples were stained with hematoxylin and eosin (HandE) and Masson Trichrome (MT), then the capillary proliferation, inflammation, necrosis, fibroblast proliferation, and fibrosis parameters were examined (×200). The capillary proliferation values were stated numerically by counting. Inflammation (neutrophil infiltration) was graded as mild (1 point), moderate (2 points), or severe (3 points). In the necrosis parameter, complete epidermal and dermal organization was scored as 1, moderate epidermal and dermal organization (erosive) as 2 and little epidermal and dermal organization (necrotic) as 3. In fibroblast proliferation, fine granulation tissue was categorized as 1 point, moderate granulation tissue as 2 points, and thick granulation tissue as 3 points. In the MT histochemical staining, the presence of irregular collagen fibres (fibrosis) was graded as 1 point, moderate as 2 points, and severe as 3 points.

Figure 2. Calculation of the flap total area and necrotic area using ImageJ 1.53 program.

RIPK-1 (1/100 dilution, 24 mins, ABCAM, ab106393) was examined immunohistochemically in compliance with validated procedures using a Roche Diagnostic Ventana Benchmark XT staining device. A brown colour showing the presence of antibody-dependent antigen was examined under a light microscope (Olympus Corporation, Tokyo, Japan) (x200) fitted with a computer-controlled digital camera and imaging software and the RIPK-1 (+) stained cell percentage was determined for each sample.

Before the rats were sacrificed, a 1.5 cc cardiac blood sample was taken. The samples were centrifuged at 1000 rpm for 15 mins and plasma was obtained. The samples obtained were evaluated using the Elabscience rat TNF-α kit according to the protocol defined by the manufacturer.

2.5. Statistical Analysis

Data obtained in the study were analyzed statistically using SPSS vn. 28.0 software. Conformity of the data to normal distribution was assessed using the Shapiro-Wilk and Kolmogorov-Smirnov tests. In the comparisons of three groups of independent variables showing normal distribution, the One-Way ANOVA test was used. In the post-hoc tests of the 3-group comparisons where a significant difference was determined, the LSD test was used when variances showed homogenous distribution and the Tamhane T2 test when not homogenous. A value of $P<0.05$ was accepted as statistically significant.

3. Results

When examined according to the groups, the mean macroscopic necrotic area was determined to be 41.36% in Group 1 and 40.66% in Group 2 as the control groups, followed by the drug groups as 36.56% in Group 3 and 31.67% in Group 4. Although the percentages were lower in the necrostatin-1 group (Group 4) and the enoxaparin group (Group 3), no statistically significant difference was determined between the groups ($P>0.05$) (Table 1). The inflammation scores were calculated for all the groups, as 2.375 for Group 1 and 2.250 for Group 2 as the control groups, and 2.000 for Group 3 and 1.625 for Group 4 as the drug groups. The values of Groups 3 and 4 were lower but in the statistical analysis, the difference was not significant ($F=2.649$, $P>0.05$).

The capillary proliferation values were determined as mean 15.375 and 22.375 for Group 1 and Group 2, respectively, and 37.0 and 47.75 for Group 3 and Group 4, respectively. The difference between the groups was determined to be statistically significant ($F=16.330$, $P<0.05$). Post-hoc paired comparisons were performed to determine from which groups the difference originated (Table 2).

Table 1. Comparisons of the necrosis percentages of the groups

		N	Mean (%)	SD*	95% CI		F	P*
					Min	Max		
Necrosis percentage	Group 1	8	41.362	8.571	34.196	48.528	1.687	.192
	Group 2	8	40.662	13.511	29.367	51.958		
	Group 3	8	36.562	6.149	31.421	41.703		
	Group 4	8	31.675	9.147	24.027	39.322		
	Total	32	37.565	10.032	33.948	41.182		

*SD= standart deviations. P value <0.05 was accepted as statistically significant. Group 1= no tratment group, Group 2= dmso + isotonic group, group 3= enoxparin group and group 4= necrostatin-1 group.

Table 2. Post-hoc analyses of the capillary proliferation counts of the groups*

	(I) Groups	(J) Groups	Mean difference (I-J)	SH	P	95% CI	
						Min	Max
Capillary proliferation	Group 1	Group 2	-7.00000	5.08960	.180	-17.4256	3.4256
		Group 3	-21.62500	5.08960	<.001	-32.0506	-11.1994
		Group 4	-32.37500	5.08960	<.001	-42.8006	-21.9494
	Group 2	Group 1	7.00000	5.08960	.180	-3.4256	17.4256
		Group 3	-14.62500	5.08960	.008	-25.0506	-4.1994
		Group 4	-25.37500	5.08960	<.001	-35.8006	-14.9494
	Group 3	Group 1	21.62500	5.08960	<.001	11.1994	32.0506
		Group 2	14.62500	5.08960	.008	4.1994	25.0506
		Group 4	-10.75000	5.08960	.044	-21.1756	-.3244
	Group 4	Group 1	32.37500	5.08960	<.001	21.9494	42.8006
		Group 2	25.37500	5.08960	<.001	14.9494	35.8006
		Group 3	10.75000	5.08960	.044	.3244	21.1756

*In the post-hoc paired comparisons of the groups, the capillary proliferation counts recorded for Group 3 and Group 4 were determined to be statistically significantly higher than the values of Group 1 (P<0.001) and Group 2 (P<0.001). In the comparison of the two drug groups, the capillary proliferation value of Group 4 was determined to be significantly higher than that of Group 3 (P: 0.044). CI= confidence interval, P value <0.05 was accepted as statistically significant.

Accordingly, the capillary proliferation counts recorded for Group 3 and Group 4 were determined to be statistically significantly higher than the values of Group 1 (P<0.001) and Group 2 (P<0.001). In the comparsion of the two drug groups, the capillary proliferation value of Group 4 was determined to be significantly higher than that of Group 3 (P=0.044). The necrosis scores were determined as mean 2.50 and 2.370 for Group 1 and Group 2, respectively, and 2.125 and 1.750 for Group 3 and Group 4, respectively (Table 3). The difference between the groups was determined to be statistically significant (F=2.2970, P<0.05). In the post-hoc paired comparisons, the necrosis score of Group 4 was determined to be significantly lower than the score of Group 1 (P=0.010) and Group 2 (P=0.029). In the comparison between Group 3 and Group 4, the mean necrosis score was observed to be lower but the difference between the groups was not statistically significant (P=0.178) (Figure 3).

Table 3. Comparisons of the necrosis scores of the groups*

		N	Mean	SD	Min	Max	F	P
Necrosis	Group 1	8	2.500	.534	2.053	2.946	2.970	.049
	Group 2	8	2.370	.515	1.942	2.807		
	Group 3	8	2.125	.687	1.589	2.660		
	Group 4	8	1.750	.462	1.363	2.137		
	Total	32	2.185	.599	1.974	2.401		

*Table 3 shows the comparison of necrosis scores by groups. One-Way ANOVA test was used to compare normally distributed variables in three independent groups. Accordingly, necrosis scores showed a significant difference between groups, F=2.2970, p<0.05. P value <0.05 was accepted as statistically significant.

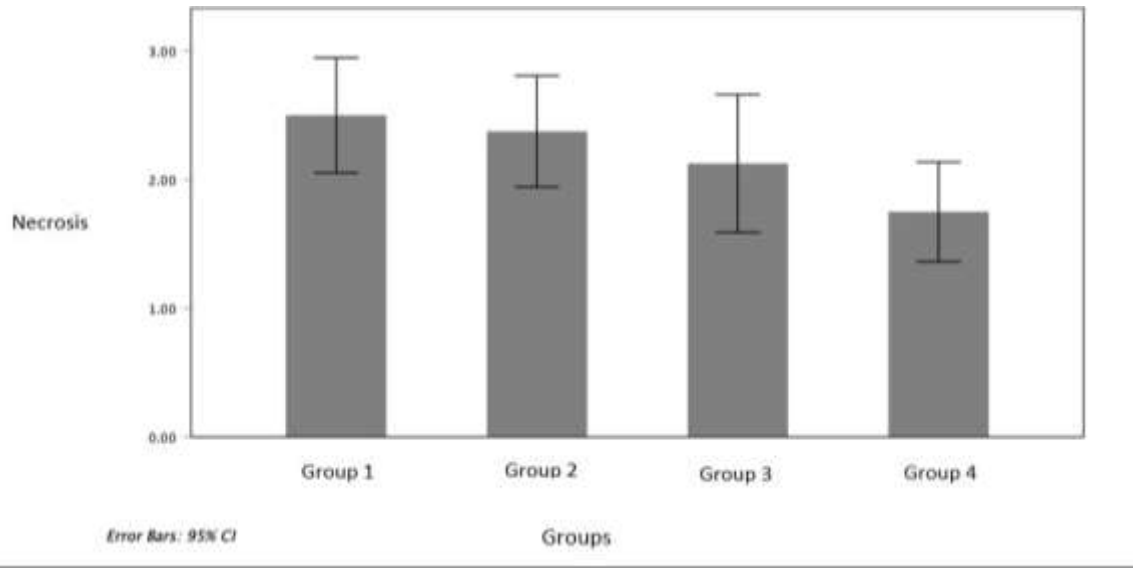

Figure 3. Comparisons of the necrosis scores of the groups. The necrosis scores were determined as mean 2.50 and 2.370 for Group 1 and Group 2, respectively, and 2.125 and 1.750 for Group 3 and Group 4, respectively. In the post-hoc paired comparisons, the necrosis score of Group 4 was determined to be significantly lower than the score of Group 1 (P=0.010) and Group 2 (P=0.029). In the comparison between Group 3 and Group 4, the mean necrosis score was observed to be lower but the difference between the groups was not statistically significant (P=0.178).

The fibroblast proliferation scores were determined as mean 1.375 and 1.625 for Group 1 and Group 2, respectively, and 2.500 and 2.375 for Group 3 and Group 4, respectively. The difference between the groups was determined to be statistically significant (F=5.896, P<0.05). In the post-hoc paired comparisons of the groups, the fibroblast proliferation scores of Group 3 and Group 4 were determined to be statistically significantly higher compared to Group 1 and Group 2 (P<0.05) (Table 4).

Table 4. Post-hoc analyses of the fibroblast proliferation scores of the groups

	(I) Groups	(J) Groups	Mean difference (I-J)	SH	P	Min	Max
Fibroblast proliferation	Group 1	Group 2	-.25000	.32217	.444	-.9099	.4099
		Group 3	-1.12500	.32217	.002	-1.7849	-.4651
		Group 4	-1.00000	.32217	.004	-1.6599	-.3401
	Group 2	Group 1	.25000	.32217	.444	-.4099	.9099
		Group 3	-.87500	.32217	.011	-1.5349	-.2151
		Group 4	-.75000	.32217	.027	-1.4099	-.0901
	Group 3	Group 1	1.12500	.32217	.002	.4651	1.7849
		Group 2	.87500	.32217	.011	.2151	1.5349
		Group 4	.12500	.32217	.701	-.5349	.7849
	Group 4	Group 1	1.00000	.32217	.004	.3401	1.6599
		Group 2	.75000	.32217	.027	.0901	1.4099
		Group 3	-.12500	.32217	.701	-.7849	.5349

*In the post-hoc paired comparisons of the groups, the fibroblast proliferation scores of Group 3 and Group 4 were determined to be statistically significantly higher compared to Group 1 and Group 2 (P<0.05). P value <0.05 was accepted as statistically significant.

The fibrosis scores were determined as mean 2.370 and 1.750 for Group 1 and Group 2, respectively, and 2.375 and 1.375 for Group 3 and Group 4, respectively. The difference between the groups was determined to be statistically significant (F=4.903, P<0.05).

The RIPK-1 immunohistochemically staining percentages were determined as mean 53.75% and 55.525% for Group 1 and Group 2, respectively, and 61.250% and 21.875% for Group 3 and Group 4, respectively. In the post-hoc paired comparisons of the groups, the RIPK-1 staining percentage of Group 4 was determined to be statistically significantly higher compared to Group 1 (P= 0.002), Group 2 (P=0.003), and Group 3 (P<0.001) (P<0.05) (Figure 4).

The descriptive statistics of the variables examined are shown as mean and standard deviation values in Table 5.

No statistically significant difference was determined between the groups in respect of the TNF-α levels (F=1.759, P>0.05).

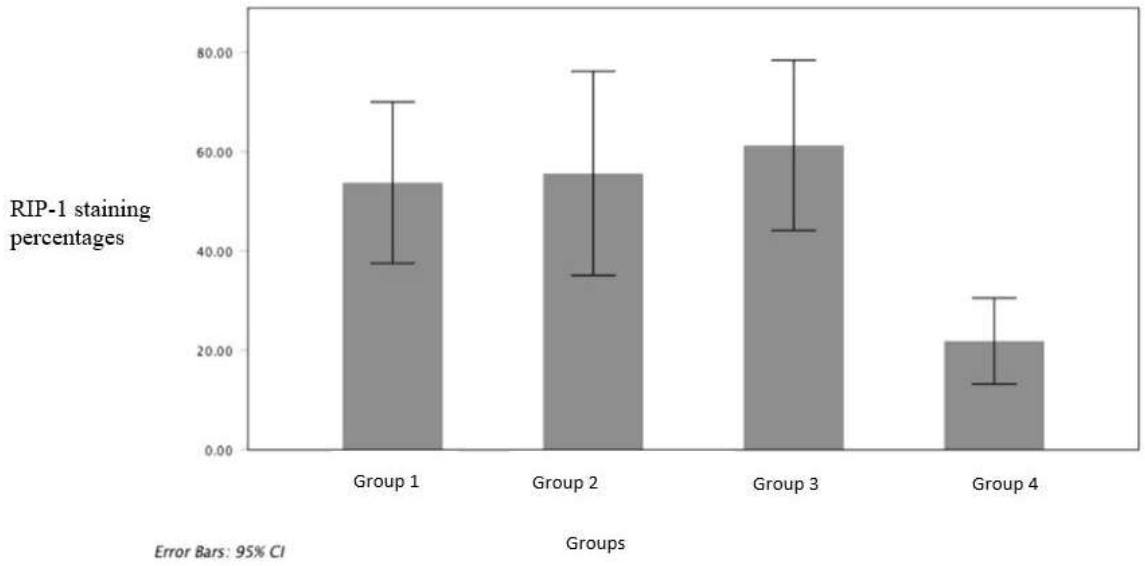

Figure 4. Comparisons of the RIP-1 staining percentages of the groups. The RIPK-1 immunohistochemically staining percentages were determined as mean 53.75% and 55.525% for Group 1 and Group 2, respectively, and 61.25% and 21.875% for Group 3 and Group 4, respectively. In the post-hoc paired comparisons of the groups, the RIPK-1 staining percentage of Group 4 was determined to be statistically significantly higher compared to Group 1 (P= 0.002), Group 2 (P=0.003), and Group 3 (P<0.001) (P<0.05).

Table 5. Descriptive findings of the variables*

	Groups							
	Group 1		Group 2		Group 3		Group 4	
	Mean	SD*	Mean	SD	Mean	SD	Mean	SD
Necrosis area percentage	41.36	8.57	40.66	13.51	36.56	6.15	31.67	9.15
Capillary proliferation	15.37	1.69	22.38	10.91	37.00	13.49	47.75	10.51
Inflammation	2.38	.52	2.25	.71	2.00	.53	1.63	.52
Necrosis	2.50	.53	2.38	.52	2.13	.64	1.75	.46
Fibroblast proliferation	1.38	.52	1.63	.52	2.50	.76	2.38	.74
Fibrosis	2.38	.74	1.75	.71	2.38	.52	1.38	.52
RIP-1 staining percentage	53.75	19.41	55.63	24.56	61.25	20.49	21.88	10.33
TNF-alpha level	17.89	7.21	20.91	12.43	16.44	7.74	11.66	1.96

*Descriptive statistics of the studied variables are shown in Table 5. Accordingly, the means and standard deviations of the variables are shown. SD= standard deviations.

4. Discussion

In this study of a flap necrosis model, two molecules were examined. The study results demonstrated that significantly better results were obtained, especially in the necrostatin-1 group, in respect of necrosis percentage, capillary proliferation, necrosis scores, fibroblast proliferation, and fibrosis scores.

In a study by Koudstaal et al. (2015) the effects of nec-1 molecule were examined on I/R damage formed in pig myocardium. Groups administered 1mg/kg and 3.3 mg/kg nec-1 was compared with a control group. The results showed that the infarct area of the group administered 1 mg/kg nec-1 was less but the difference was not statistically significant, whereas in the group administered 3.3 mg/kg nec-1, the infarct area developing associated with I/R damage was seen to be statistically significantly less compared to the control group.

In the current study, no statistically significant difference was observed in the flap necrosis percentage of the nec-1 group (Group 4) compared to the control groups of Group 1 and Group 2. However, the flap necrosis percentage of the nec-1 group (31.675%) was observed to be evidently lower than that of the other two groups (41.36%, 40.66%, respectively). The drug dose in the current study was determined as 1.65 mg/kg/day (Liu et al., 2019b). In the Koudstaal et al. (2015) study, the use of 3.3mg/kg nec-1 was shown to be more effective than the dose of 1mg/kg in inhibiting necroptotic cell death and decreasing the tissue infarctus area. In the light of these data, it can be considered that the use of a higher dose of nec-1 in a random pattern flap model will increase flap viability more than the use of a low dose.

Neonatal hypoxic-ischaemic rat model, nec-1 treatment was seen to decrease neuro-inflammation and inhibit NF-KB activation and IL-1β, IL-6, and TNF-α expression by blocking the interaction of RIPK-1 and RIPK-3 in neurons. As a result of that study, it was seen that nec-1 could have a protective effect against hypoxic-ischaemic brain damage in newborns (Chavez-Valdez, et al., 2012; Azboy et al., 2014; Cao and Mu, 2021;). Wen et al. (2017) conducted an experimental study examining the effects of nec-1 molecule on intestinal I/R damage, and as a result of that study, it was found that the intestinal mucosal structure of the animals in the group administered nec-1 molecule (1.0 mg/kg) was significantly protected and they had lower mucosal damage scores compared to the control group. In the current study, the tissue RIPK-1 staining levels were compared immunohistochemically, and consistent with similar studies in the literature, RIPK-1 (+) stained cells were determined at a high rate (53.75%) in the control group, and the percentage of RIPK-1 (+) stained cells was observed to be significantly low (21.87%) in the rats administered nec-1.

There are different results in literature related to the use of enoxaparin molecule in a random pattern flap model (Miyawaki et al., 2002; Chung et al., 2006). Fatemi et al. (2012) investigated the effects of enoxaparin and clopidogrel molecules in a random pattern skin flap model and the results of the study showed no statistically significant difference between the enoxaparin molecule and the control group in reducing the flap necrosis percentage. In contrast, Aral et al. (2015) reported that enoxaparin molecule was effective in reducing flap necrosis and increasing flap viability in a random pattern flap model. In Group 3 of the current study, administered with 100 IU/kg enoxaparin, the flap necrosis percentage was determined to be 36.56%. Although this rate was lower than that of Group 1 (41.36%) and Group 2 (40.66%), no statistically significant difference was determined between the groups. These results in the current study are not consistent with the findings of the similar study by Aral et al. This difference could be due to the fact that Aral et al. administered a dose of 320 IU/kg enoxaparin molecule subcutaneously directly into the flap tissue (Aral et al., 2015).

When the flap necrosis percentages were compared between the nec-1 and enoxaparin groups, the flap necrosis percentages were observed to be 31.675% and 36.56%, respectively. Although the necrosis percentage was lower in the nec-1 group, the difference was not found to be statistically significant.

All the groups in the current study were compared in respect of inflammation (neutrophil inflammation), capillary proliferation, fibroblast proliferation, fibrosis and necrosis parameters. No statistically significant difference was determined in respect of the inflammation scores, but the mean inflammation score of the nec-1 group was determined to be lower than that of both the enoxaparin group and the control groups, Groups 1 and 2. Previous studies in literature have shown the anti-inflammatory effect of both nec-1 molecule and enoxaparin molecule (Iba and Miyasho, 2008; Iba et al., 2012; Iba et al., 2013; Wen et al., 2017). When the necrosis scores were compared between the two drug groups, lower scores were observed in the nec-1 group but the difference was not statistically significant. These histopathological results suggest that there are positive effects of nec-1 molecule on tissue healing in a flap necrosis model, but when the data of the enoxaparin group were examined, this effect was seen to be limited.

A limitation of this study was that only a single dose of nec-1 molecule was administered. The administration of different doses could provide clearer information for the interpretation of the data in the examination of a flap necrosis model.

There is a need for further research of more extensive animal groups to investigate the effects of different doses and potential side-effects of necrostatin, which is an experimental molecule. Moreover, there are different results in literature related to the effect of the enoxaparin molecule in random pattern flaps. When the flap necrosis percentage and histochemical parameters of the enoxaparin molecule were evaluated in this study, the effects on flap viability were considered to be limited. Further studies of enoxaparin molecule with larger samples and drugs used at different doses and applications would be helpful in clarifying the different results in literature.

5. Conclusion

When the flap necrosis percentages and immunohistochemical and histopathological data obtained in this study are taken into consideration, they suggest that necrostatin molecule has significant therapeutic potential for increasing flap viability in the random pattern flap model. When the flap necrosis percentage and histochemical parameters of the enoxaparin molecule were evaluated in this study, the effects on flap viability were considered to be limited.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	Ö.F.Ç.	N.B.	E.K.G.	M.S.	İ.O.	M.G.Y.
C	40	20	20	20		
D	50	25			25	
S	100					
DCP	40	20			20	20
DAI			50	50		
L	50	20				30
W	50	25				25
CR	20	20	20	20	20	
SR	50					50
PM	20	40			20	20
FA	50	50				

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Acknowledgments

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Ethical Approval/Informed Consent

All the study protocols were approved by the Animal Experiments Ethics Committee of Kahramanmaras Sutcu Imam University Medical Faculty (approval date: October 13, 2021, protocol code: 18-2021/07-01). All procedures were in compliance with the National Health Institutes regulations for the use and care of laboratory animals.

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