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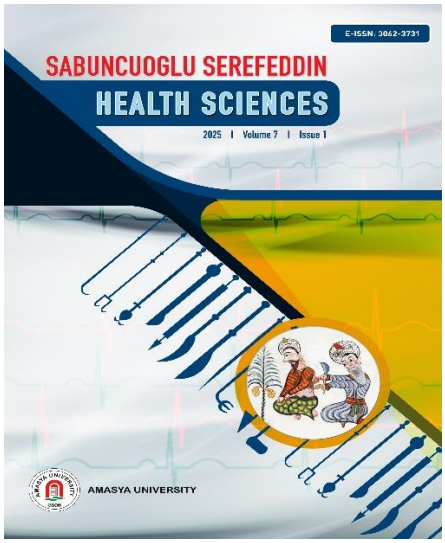

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IN SILICO TOXIC EFFECTS OF 2-(4-TERT-BUTYLPHENYL) PROPANAL, 3-(4-TERT-BUTYLPHENYL)-2-METHYLPROPANAL AND LILAL MOLECULES AND PREGNANCY

2-(4-TERT-BUTİLFENİL) PROPANAL, 3-(4-TERT-BUTİLFENİL)-2-METİLPROPANAL VE LİLAL MOLEKÜLLERİNİN IN SILICO TOKSİK ETKİLERİ VE GEBELİK

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Research Article
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Abstract
In our daily lives, we are exposed to many chemicals originating from products such as cosmetics, cleaning products, and food. These substances may be potential carcinogens and endocrine disruptors. In recent years, BMHCA (3-(4-(tert-butyl) phenyl)-2-methylpropanal, Lilial) and its derivatives have attracted much attention. These substances are generally used in products for fragrance purposes. BMHCA has been published in the EU Cosmetic Products Regulation and banned in cosmetic products offered in the EU and Northern Ireland markets. In this study, the toxic effects of 2-(4-tert-butylphenyl) propanal, 3-(4-tert-butylphenyl)-2-methylpropanal, and Lilal were determined by in silico analysis. According to the results obtained, it was determined that the molecules had moderate and high toxicity levels. The common features of all these compounds include their ability to cross the blood-brain barrier (BBB) at a high rate and their potential to be moderately to highly carcinogenic. Our study is a preliminary study and needs to be supported by in vitro and in vivo studies.

Keywords: BMHCA(Lilial), Carcinogens, Obstetrics and Gynaecology, Pregnancy, Toxicity.

Öz
Günlük hayatımızda kozmetik, temizlik, gıda gibi ürünler kaynaklı pek çok kimyasal maddeye maruz kalmaktayız. Bu maddeler, potansiyel kanserojen ve endokrin bozucu olabilirler. Son yıllarda BMHCA (3-(4-(tert-butil) fenil)-2-metilpropanal, Lilial) ve türevleri çok dikkat çekmektedir. Bu maddeler genellikle koku verme amacıyla ürünlerde kullanılmaktadır. BMHCA, AB Kozmetik Ürünler Yönetmeliği'nde yayımlanarak AB ve Kuzey İrlanda pazarında sunulan kozmetik ürünlerde yasaklanmıştır. Bu çalışmada 2-(4-tert-butilfenil) propanal, 3-(4-tert-butilfenil)-2-metilpropanal ve Lilal'ın toksik etkileri in silico analiz ile belirlenmiştir. Elde edilen sonuçlara göre, moleküllerin orta ve yüksek toksisite düzeylerine sahip olduğu belirlenmiştir. Tüm bu bileşiklerin ortak özellikleri arasında kan-beyin bariyerini (BBB) yüksek oranda geçebilme ve orta-yüksek düzeyde kanserojen olma potansiyeli de yer almaktadır. Bizim çalışmamız, ön çalışma niteliği taşımakta olup in vitro ve in vivo çalışmalarla desteklenmesi gerekmektedir.

Anahtar Kelimeler: BMHCA (Lilial), Karsinojenler, Kadın Doğum, Gebelik, Toksisite.

1. Introduction

The widespread and increasing use of chemical products in modern daily life has raised significant concerns regarding their potential adverse effects on maternal health and fetal development during pregnancy. Products such as hand and face creams, deodorants, hair dyes, nail polishes, sunscreens, cream-based deodorants, and colognes—particularly those formulated with synthetic floral fragrances—constitute major sources of exposure to potentially harmful chemicals. In particular, the frequent use of colognes has markedly intensified following the global COVID-19 pandemic, further increasing the risk of exposure during pregnancy (Özdemir et al., 2022). The escalating use of chemical products in everyday life has emerged as a significant public health concern,

particularly regarding maternal and fetal well-being during pregnancy. Among these products are hand and face creams, deodorants, hair dyes, nail polishes, sunscreens, cream-based deodorants, and colognes, with particular concern surrounding colognes containing synthetic floral fragrances. Following the onset of the COVID-19 pandemic, the widespread use of colognes has markedly increased, further elevating the potential for chemical exposure among pregnant women (Özdemir et al., 2022).

Table 1: Cosmetic Products (Sade & Özkan, 2020)

Cosmetic Products	Application Area	Purpose of Use	Product Structure
• Applied to the outer part of the human body	• Applied to the skin	• Cleansers	• Solutions
• Applied to hair, fibers, and nails	• Applied to external genital organs	•Moisturizers and emollients	• Suspensions
• Applied to teeth and oral cavity	• Anti-wrinkle agents	• Nourishers	• Emulsions
	• Spot removers	• Tanning agents	• Creams
	• Sunscreens	• Baby cosmetics	• Pastes
			• Gels
			• Powders

Pregnancy is a period characterized by tightly coordinated hormone-mediated events that cause changes in maternal physiology to adapt to the developing fetus, prepare for childbirth, and facilitate breastfeeding. During this time, dramatic changes in the mother's metabolism, reproductive organs, endocrine activity, and immune system increase sensitivity to chemical substances. The literature has reported serious side effects and diseases associated with exposure to chemicals and their impacts on women's health (ACOG Committee Opinion No. 575; Varshavsky et al., 2020; Wang et al., 2016).

Endocrine-disrupting chemicals (EDCs) can enhance biological changes during pregnancy and affect hormone levels by mimicking or blocking cell signaling and interfering with hormone production or degradation (Gore et al., 2015). EDCs are commonly found in consumer and personal care products; however, safety oversight in the United States is limited. It has been reported that EDCs and other chemicals contribute to a high body burden among pregnant women and children in the U.S. (Hendryx et al., 2018; Varshavsky et al., 2020).

Flavors and fragrances are defined as substances that directly stimulate taste and smell receptors in the mouth and nose. They are incorporated into many pharmaceutical and personal care products (PPCPs) — including perfumes, creams, lotions, detergents, and various personal and household products — to enhance or alter their scents and/or flavors and are released into the environment as pollutants due to human activities (Di Sotto et al., 2014).

The number of aromatic compounds used in everyday products is quite extensive. The Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) has a list containing 2,750 entries for fragrance and aromatic raw materials. The European Food Safety Authority (EFSA) has reported a list of compounds for which additional toxicity data is required to identify safely usable fragrances in food materials (EFSA, 2011; Di Sotto et al., 2014).

Among these compounds, aldehydes are defined as a group of potentially reactive substances due to the presence of polarized carbon-oxygen double bonds in their structures. Because of their reactivity, certain aldehydes can interact with electron-rich biological macromolecules, leading to adverse health effects such as general toxicity, allergic reactions, genotoxicity, and carcinogenicity (Langton et al., 2006).

BMHCA(Lilial), used as a synthetic fragrance in cosmetic products and recently banned in the EU, falls under the aldehyde group. Commonly used chemical substances in daily life include perfumes, colognes, cosmetic products, and cleaning agents. BMHCA (3-(4-(tert-butyl)phenyl)-2-methylpropanal) is utilized in these products as a synthetic fragrance. This substance is also known as lyal, lilac, and lily aldehyde. Today, its use has been banned in most countries due to its classification as a strong allergen (skin sensitizer) and reproductive toxicant (CMR1B classification) (ECHA, 2023; Jablonská et al., 2023).

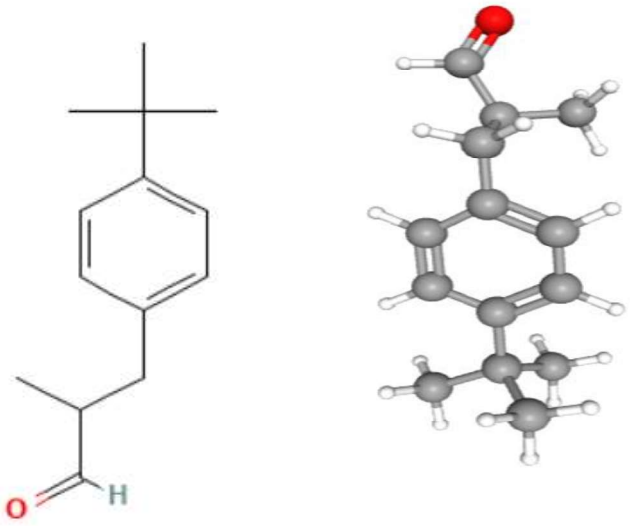

Figure 1. Chemical Structure of 3-(4-(tert-butyl)phenyl)-2-methylpropanal

Lilial®, also known as BPMP and 2-4-tert-butylphenylpropionaldehyde, is a compound within the aldehyde group that includes a structure prone to oxidation and is responsible for cell and DNA damage. It is chemically characterized by a floral scent and is used as an ingredient in personal care and household products, as well as an additive in the food and pharmaceutical industries (Di Sotto et al., 2014; Jablonská et al., 2023).

Hydroxycinnamic aldehydes (HCA) and p-tert-butyl-alpha-methylhydrocinnamic aldehyde (BMHCA) are widely used as flavoring compounds in medical and consumer products, food, beverages and desserts. Chemically, these compounds are synthetic aldehydes characterized by carbonyl groups containing polarized carbon-oxygen double bonds. The electronegativity difference between the oxygen and carbon atoms in the carbonyl group allows these compounds to react with electron-rich biomolecules such as DNA and proteins, thereby causing adverse health effects such as toxicity, allergenic reactions, mutagenicity and carcinogenicity (Garaycoechea et al., 2012). In addition, BMHCA has been reported to pose a potential health risk due to its metabolism to a reactive α,β-unsaturated intermediate (Usta et al., 2013; Di Sotto et al., 2014).

Studies have shown that HCA and BMHCA do not cause genotoxic effects. In the presence of an exogenous metabolic activation system, a lack of point mutations such as frameshifts and oxidative damage was observed in bacteria, indicating that a genotoxic derivative was not produced during CYP450-mediated biotransformations. In some cases, the presence of the metabolic activator reduced the toxicity of the tested substances. No damage was observed at the chromosomal level in mammalian cells (Di Sotto et al., 2014).

In vitro studies report that lilial does not exhibit genotoxicity and mutagenicity. It has been reported that it does not carry genotoxic and mutagenic potential in bacteria such as S. typhimurium, E. coli, CHO(Chinese hamster ovary) cell and Chinese hamster V79 cells (Bernauer et al., 2019). At concentrations up to 500 μM, lilialin did not cause genotoxicity (clastogenicity or aneuploidy) or DNA strand breaks at the chromosomal level (Di Sotto et al., 2014). However, in a study conducted in fertilized eggs of white turkeys (Meleagris gallopavo) in vivo, lilialin caused significant DNA breaks in the comet test (2.0-fold) (Kobets et al., 2018; Jablonská et al., 2023). The endocrine disrupting effects of lilialin have been reported by the European Chemicals Agency (ECHA, 2023). In addition, lilialin has been shown to exhibit estrogenic activity in MCF-7 human breast cancer cells (Charles and Darbre, 2009). However, the effects of lilialin in some sensitive species are thought to be due to toxicity in seminiferous tissues rather than endocrine disrupting effects. Lilialin has also been shown to cause eye and skin irritation in rabbits in various studies (Bernauer et al., 2019; Jablonská et al., 2023). Dermal absorption rates were determined as 13.5% for hydroalcoholic-based fragrances and deodorant/antiperspirant products, 8.9% for oil-in-water-based products, and 10.5% for water-in-oil-based products. Lilialin has been reported to cause skin sensitization reactions at a concentration of 5% (Api et al., 2020; Lalko et al., 2004; Roberts et al., 2007). In an in vitro study with Caco-2 cells, lilialin was reported to be 80% recovered by Caco-2 cells with high solubility and low metabolism (Jablonská et

al., 2023). Lilialin was observed to significantly reduce relative viability in HeLa9903 cells at a concentration of 100 nM. However, the relative viability above 80% at all concentrations (up to 100 µM) indicates that lilial is not cytotoxic (Jablonská et al., 2023).

The European Union initially restricted the use of lilial due to its skin sensitizing properties. Lilial concentrations exceeding 0.001% in skin-retaining products and 0.01% in washable products must be declared on product labels. In 2022, the European Union banned the use of lilial in cosmetic products due to its CMR 1B classification (reproductive health hazardous substance). However, there are several studies in the literature that provide contradictory and inconsistent findings on the toxicological effects of lilial (Jablonská et al., 2023).

Lilial is identified as one of the 26 fragrance ingredients that cause allergic contact dermatitis and must be declared as an ingredient in cosmetic products (Heisterberg et al., 2011).

2. Material and Methods

A comprehensive toxicity assessment study was conducted to investigate the potential toxic effects of Lilial and its structurally similar analog compounds. In this study, the freely accessible online tool available at https://tox.charite.de/ was employed to perform detailed toxicity predictions. To facilitate the analysis, the SMILES (Simplified Molecular Input Line Entry System) notations, which provide a standardized way to represent chemical structures in text form, were retrieved for Lilial and its analog compounds from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Using these SMILES strings, the toxicity profiles of three selected compounds were comparatively examined. Various toxicological parameters, including mutagenicity, carcinogenicity, and potential for skin sensitization, were evaluated and compared across the compounds. This approach provided valuable insights into the toxicological characteristics of Lilial and its analogs, contributing to a better understanding of their potential risks for human health and environmental safety.

3. Results and/or Discussion

In the conducted study, the in silico toxicity of the molecules 2-(4-tert-butylphenyl) propanal, 3-(4-tert-butylphenyl)-2-methylpropanal, and Lilial was investigated.

In the toxicity assessment study conducted on 2-(4-tert-Butylphenyl) propanal using the website https://tox.charite.de/, it was determined that this compound is a moderately to highly toxic substance with a toxicity level of 5 (Figure 2). The estimated LD50 dose was found to be 3500 mg/kg. Additionally, it was identified that 2-(4-tert-Butylphenyl) propanal possesses neurotoxic and carcinogenic properties, and is also a substance that can cross the blood-brain barrier (BBB) (Table 2).

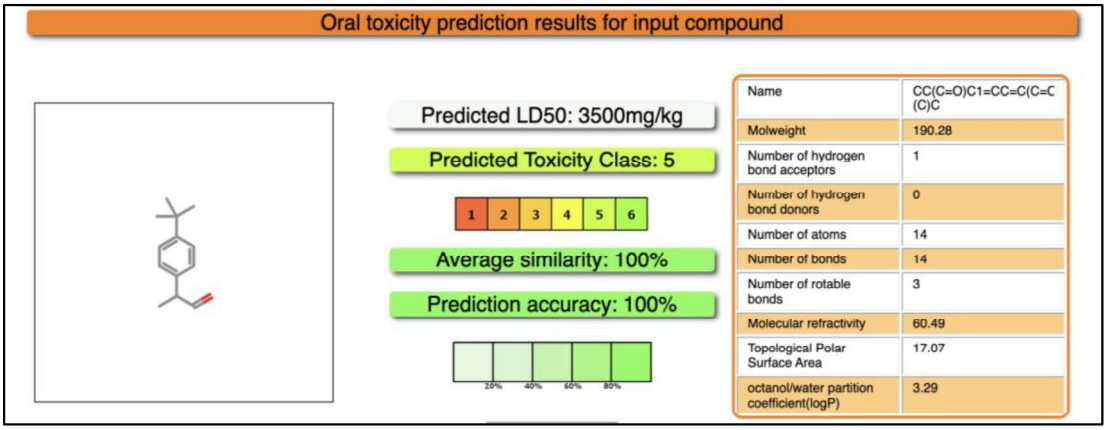

Figure 2. (4-tert-Butylphenyl) propanal

Table 2. Toxicity Results of 2-(4-tert-Butylphenyl) propanal

Toxicity Model Report

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Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Inactive	0.76
Organ toxicity	Neurotoxicity	neuro	Active	0.54
Organ toxicity	Nephrotoxicity	nephro	Inactive	0.88
Organ toxicity	Respiratory toxicity	respi	Inactive	0.86
Organ toxicity	Cardiotoxicity	cardio	Inactive	0.77
Toxicity end points	Carcinogenicity	carcino	Active	0.54
Toxicity end points	Immunotoxicity	immuno	Inactive	0.97
Toxicity end points	Mutagenicity	mutagen	Inactive	0.94
Toxicity end points	Cytotoxicity	cyto	Inactive	0.87
Toxicity end points	BBB-barrier	bbb	Active	0.99
Toxicity end points	Ecotoxicity	eco	Active	0.67
Toxicity end points	Clinical toxicity	clinical	Inactive	0.74
Toxicity end points	Nutritional toxicity	nutri	Inactive	0.93
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	1.0
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	1.0
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	1.0
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.84
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Binding Domain (ER-LBD)	nr_er_lbd	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	1.0
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	1.0
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	1.0
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.99
Tox21-Stress response pathways	Phosphoprotein (Tumor Supressor) p53	sr_p53	Inactive	1.0
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	Inactive	1.0
Molecular Initiating Events	Thyroid hormone receptor alpha (THRα)	mie_thr_alpha	Inactive	0.93
Molecular Initiating Events	Thyroid hormone receptor beta (THRβ)	mie_thr_beta	Inactive	0.94
Molecular Initiating Events	Transtyretrin (TTR)	mie_ttr	Active	0.51
Molecular Initiating Events	Ryanodine receptor (RYR)	mie_ryr	Inactive	0.97
Molecular Initiating Events	GABA receptor (GABAR)	mie_gabar	Inactive	0.53
Molecular Initiating Events	Glutamate N-methyl-D-aspartate receptor (NMDAR)	mie_nmdar	Inactive	0.95
Molecular Initiating Events	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor (AMPAR)	mie_ampar	Inactive	1.0
Molecular Initiating Events	Kainate receptor (KAR)	mie_kar	Inactive	1.0
Molecular Initiating Events	Achetylcholinesterase (AChE)	mie_ache	Active	0.63
Molecular Initiating Events	Constitutive androstane receptor (CAR)	mie_car	Inactive	0.99
Molecular Initiating Events	Pregnane X receptor (PXR)	mie_pxr	Active	0.52
Molecular Initiating Events	NADH-quinone oxidoreductase (NADHOX)	mie_nadhox	Inactive	0.85
Molecular Initiating Events	Voltage gated sodium channel (VGSC)	mie_vgsc	Inactive	0.88
Molecular Initiating Events	Na+/I- symporter (NIS)	mie_nis	Inactive	0.97
Metabolism	Cytochrome CYP1A2	CYP1A2	Inactive	0.93
Metabolism	Cytochrome CYP2C19	CYP2C19	Inactive	0.65
Metabolism	Cytochrome CYP2C9	CYP2C9	Active	0.53
Metabolism	Cytochrome CYP2D6	CYP2D6	Inactive	0.85
Metabolism	Cytochrome CYP3A4	CYP3A4	Inactive	0.94
Metabolism	Cytochrome CYP2E1	CYP2E1	Inactive	0.97

In the toxicity study conducted on 3-(4-Tert-butylphenyl)-2-methylpropanal, it was determined that this compound is a level 4 toxic substance (Figure 3). The estimated LD50 dose was calculated to be 2000 mg/kg. Additionally, it was found that 3-(4-Tert-butylphenyl)-2-methylpropanal can cross the blood-brain barrier (BBB) and is an ecotoxic substance. This compound also plays an active role on the cytochrome CYP2C9 enzyme (Table 3).

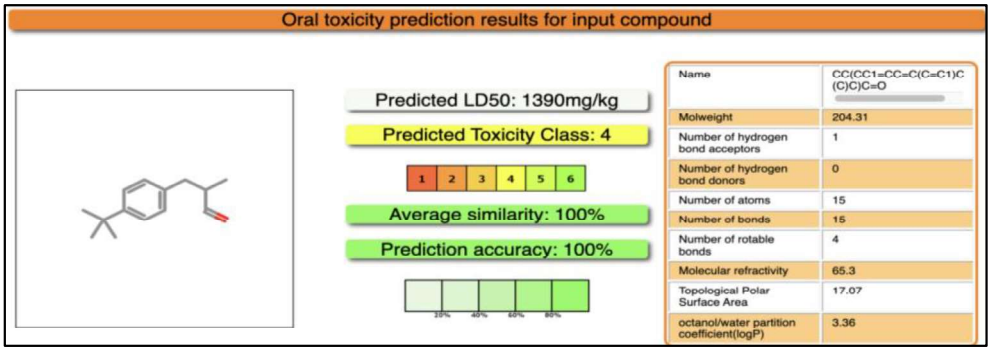

Figure 3. Chemical Structure and Properties of 3-(4-Tert-butylphenyl)-2-methylpropanal

Table 3. 3-(4-Tert-butylphenyl)-2-methylpropanal toxicity report.

Toxicity Model Report

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Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Inactive	0.69
Organ toxicity	Neurotoxicity	neuro	Inactive	0.53
Organ toxicity	Nephrotoxicity	nephro	Inactive	0.88
Organ toxicity	Respiratory toxicity	respi	Inactive	0.82
Organ toxicity	Cardiotoxicity	cardio	Inactive	0.77
Toxicity end points	Carcinogenicity	carcino	Active	0.53
Toxicity end points	Immunotoxicity	immuno	Inactive	0.99
Toxicity end points	Mutagenicity	mutagen	Inactive	0.96
Toxicity end points	Cytotoxicity	cyto	Inactive	0.86
Toxicity end points	BBB-barrier	bbb	Active	0.99
Toxicity end points	Ecotoxicity	eco	Active	0.65
Toxicity end points	Clinical toxicity	clinical	Inactive	0.77
Toxicity end points	Nutritional toxicity	nutri	Inactive	0.93
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	1.0
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	1.0
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	1.0
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.95
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Binding Domain (ER-LBD)	nr_er_lbd	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	1.0
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	1.0
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.96
Tox21-Stress response pathways	Phosphoprotein (Tumor Supressor) p53	sr_p53	Inactive	0.99
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	Inactive	1.0
Molecular Initiating Events	Thyroid hormone receptor alpha (THRα)	mie_thr_alpha	Inactive	0.89
Molecular Initiating Events	Thyroid hormone receptor beta (THRB)	mie_thr_beta	Inactive	0.94
Molecular Initiating Events	Transtyretrin (TTR)	mie_ttr	Active	0.50
Molecular Initiating Events	Ryanodine receptor (RYR)	mie_ryr	Inactive	0.97
Molecular Initiating Events	GABA receptor (GABAR)	mie_gabar	Inactive	0.52
Molecular Initiating Events	Glutamate N-methyl-D-aspartate receptor (NMDAR)	mie_nmdar	Inactive	0.93
Molecular Initiating Events	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor (AMPAR)	mie_ampar	Inactive	1.0
Molecular Initiating Events	Kainate receptor (KAR)	mie_kar	Inactive	1.0
Molecular Initiating Events	Achetylcholinesterase (AChE)	mie_ache	Inactive	0.61
Molecular Initiating Events	Constitutive androstane receptor (CAR)	mie_car	Inactive	1.0
Molecular Initiating Events	Pregnane X receptor (PXR)	mie_pxr	Inactive	0.53
Molecular Initiating Events	NADH-quinone oxidoreductase (NADHOX)	mie_nadhox	Inactive	0.84
Molecular Initiating Events	Voltage gated sodium channel (VGSC)	mie_vgsc	Inactive	0.86
Molecular Initiating Events	Na+/I- symporter (NIS)	mie_nis	Inactive	0.98
Metabolism	Cytochrome CYP1A2	CYP1A2	Inactive	0.93
Metabolism	Cytochrome CYP2C19	CYP2C19	Inactive	0.73
Metabolism	Cytochrome CYP2C9	CYP2C9	Inactive	0.50
Metabolism	Cytochrome CYP2D6	CYP2D6	Inactive	0.85
Metabolism	Cytochrome CYP3A4	CYP3A4	Inactive	0.93
Metabolism	Cytochrome CYP2E1	CYP2E1	Inactive	0.97

Investigations regarding Lilial have determined that this substance is moderately toxic (Figure 4). Our analyses conducted on the website https://tox.charite.de/ indicate that Lilial falls into toxicity class 4, with an LD50 value established at 1390 mg/kg. Furthermore, it has been identified as an

active compound that can cross the blood-brain barrier (BBB) and possesses carcinogenic properties (Table 4).

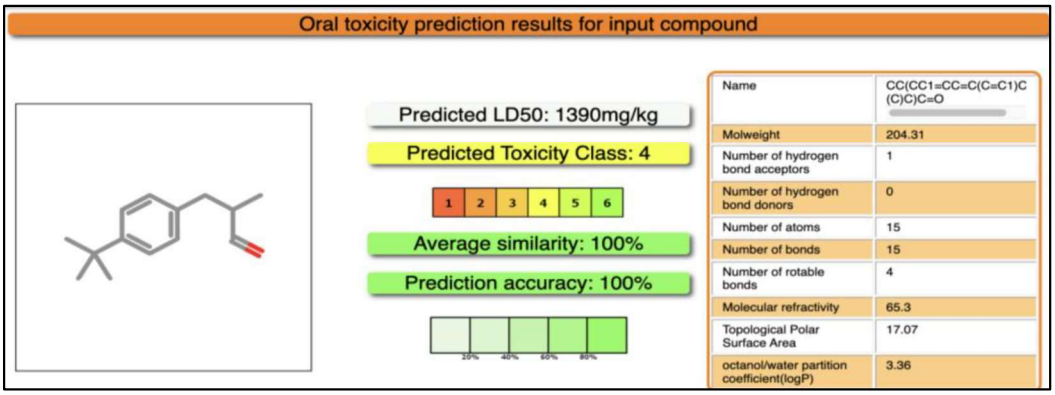

Figure 4. Chemical Structure and Properties of Lilial

Before a cosmetic product is introduced to the EU market, it is evaluated to determine its safety under foreseeable conditions of use (Regulation 1223/2009, Article 3). If the content of the cosmetic poses a risk to human health, safety must be assessed under foreseeable conditions of use. If toxicological concerns are serious for regulatory purposes, this issue is investigated by the Scientific Committee on Consumer Safety (SCCS), an independent advisory body (SCCS, 2021; Bialas, 2023). Moreover, every raw material used in cosmetics must meet the legal requirements of REACH and the Classification, Labelling, and Packaging (CLP) regulations. As of March 1, 2022, BMHCA (Lilial) has been banned in cosmetic products marketed in the EU and Northern Ireland following its publication in the EU Cosmetics Regulation (CPTA).

Hydroxycinnamic aldehydes (HCA) and p-tert-butyl-alpha-methylhydrocinnamic aldehyde (BMHCA) are widely used as flavoring compounds in medical and consumer products, as well as in food, beverages, and sweets. Chemically, these compounds are characterized as synthetic aldehydes containing polarized carbon-oxygen double bonds in carbonyl groups. The electronegativity difference between the oxygen and carbon atoms in the carbonyl group enables these compounds to react with electron-rich biomolecules such as DNA and proteins, potentially leading to adverse health effects including toxicity, allergic reactions, mutagenicity, and carcinogenicity (Garaycoechea et al., 2012). Additionally, it has been reported that BMHCA poses a potential health risk due to its metabolism into a reactive α,β-unsaturated intermediate (Usta et al., 2013; Di Sotto et al., 2014). Studies have shown that HCA and BMHCA do not cause genotoxic effects. In the presence of an exogenous metabolic activation system, a lack of point mutations, such as frameshift and oxidative damage in bacteria, was observed, indicating that no genotoxic derivatives are produced during CYP450-mediated biotransformations. In some cases, the presence of the metabolic activator has reduced the toxicity of the tested substances. Chromosomal-level damage has also not been observed in mammalian cells (Di Sotto et al., 2014).

In Vitro Studies, Reports indicate that lilialin does not exhibit genotoxicity or mutagenicity. It has been documented that it does not carry genotoxic and mutagenic potential in bacteria such as *S. typhimurium* and *E. coli*, as well as in CHO and Chinese hamster V79 cells (Bernauer et al., 2019). It has been observed that lilialin does not lead to chromosomal-level genotoxicity (clastogenicity or aneuploidy) or DNA strand breaks at concentrations up to 500 µM (Di Sotto et al., 2014). However, an in vivo study conducted on fertilized eggs of white turkey (Meleagris gallopavo) showed that lilial caused significant DNA strand breaks (2.0-fold increase) in the comet test (Kobets et al., 2018; Jablonská et al., 2023). Endocrine-disrupting effects of lilialin have been reported by the European Chemicals Agency (ECHA, 2023).

Table 4. Lilial Toxicity Report

Toxicity Model Report

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Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Inactive	0.69
Organ toxicity	Neurotoxicity	neuro	Inactive	0.53
Organ toxicity	Nephrotoxicity	nephro	Inactive	0.88
Organ toxicity	Respiratory toxicity	respi	Inactive	0.82
Organ toxicity	Cardiotoxicity	cardio	Inactive	0.77
Toxicity end points	Carcinogenicity	carcino	Active	0.53
Toxicity end points	Immunotoxicity	immuno	Inactive	0.99
Toxicity end points	Mutagenicity	mutagen	Inactive	0.96
Toxicity end points	Cytotoxicity	cyto	Inactive	0.86
Toxicity end points	BBB-barrier	bbb	Active	0.99
Toxicity end points	Ecotoxicity	eco	Active	0.65
Toxicity end points	Clinical toxicity	clinical	Inactive	0.77
Toxicity end points	Nutritional toxicity	nutri	Inactive	0.93
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	1.0
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	1.0
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	1.0
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.95
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Binding Domain (ER-LBD)	nr_er_lbd	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	1.0
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	1.0
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.96
Tox21-Stress response pathways	Phosphoprotein (Tumor Suppressor) p53	sr_p53	Inactive	0.99
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	Inactive	1.0
Molecular Initiating Events	Thyroid hormone receptor alpha (THRα)	mie_thr_alpha	Inactive	0.89
Molecular Initiating Events	Thyroid hormone receptor beta (THRß)	mie_thr_beta	Inactive	0.94
Molecular Initiating Events	Transthyretin (TTR)	mie_ttr	Active	0.50
Molecular Initiating Events	Ryanodine receptor (RYR)	mie_ryr	Inactive	0.97
Molecular Initiating Events	GABA receptor (GABAR)	mie_gabar	Inactive	0.52
Molecular Initiating Events	Glutamate N-methyl-D-aspartate receptor (NMDAR)	mie_nmdar	Inactive	0.93
Molecular Initiating Events	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor (AMPAR)	mie_ampar	Inactive	1.0
Molecular Initiating Events	Kainate receptor (KAR)	mie_kar	Inactive	1.0
Molecular Initiating Events	Acheylcholinesterase (AChE)	mie_ache	Inactive	0.61
Molecular Initiating Events	Constitutive androstane receptor (CAR)	mie_car	Inactive	1.0
Molecular Initiating Events	Pregnane X receptor (PXR)	mie_pxr	Inactive	0.53
Molecular Initiating Events	NADH-quinone oxidoreductase (NADHOX)	mie_nadhox	Inactive	0.84
Molecular Initiating Events	Voltage gated sodium channel (VGSC)	mie_vgsc	Inactive	0.86
Molecular Initiating Events	Na+/I- symporter (NIS)	mie_nis	Inactive	0.98
Metabolism	Cytochrome CYP1A2	CYP1A2	Inactive	0.93
Metabolism	Cytochrome CYP2C19	CYP2C19	Inactive	0.73
Metabolism	Cytochrome CYP2C9	CYP2C9	Inactive	0.50
Metabolism	Cytochrome CYP2D6	CYP2D6	Inactive	0.85
Metabolism	Cytochrome CYP3A4	CYP3A4	Inactive	0.93
Metabolism	Cytochrome CYP2E1	CYP2E1	Inactive	0.97

Additionally, it has been demonstrated that lilialin exhibits estrogenic activity in MCF-7 human breast cancer cells (Charles and Darbre, 2009). However, it is thought that the effects of lilialin in some sensitive species are more related to toxicity in seminiferous tissues rather than endocrine-disrupting effects. Various studies have also shown that lilialin causes eye and skin irritation in rabbits (Bernauer et al.,

2019; Jablonská et al., 2023). Dermal absorption rates have been determined as 13.5% for hydroalcoholic-based fragrances and deodorant/antiperspirant products, 8.9% for water-in-oil-based products, and 10.5% for oil-in-water-based products. It has been reported that lilialin at a 5% concentration can cause skin sensitivity reactions (Api et al., 2020; Lalko et al., 2004; Roberts et al., 2007). In an in vitro study with Caco-2 cells, it was reported that lilialin was recovered at 80% with high solubility and low metabolism (Jablonská et al., 2023). At a concentration of 100 nM, lilialin significantly reduced relative viability in HeLa9903 cells. However, the relative viability remained above 80% at all concentrations (up to 100 μM), indicating that lilialin is not cytotoxic (Jablonská et al., 2023). The European Union initially restricted the use of lilial due to its skin sensitization properties, requiring that products containing lilial at concentrations exceeding 0.001% in leave-on products and 0.01% in rinse-off products be labeled. In 2022, the European Union banned the use of lilial in cosmetic products due to its CMR 1B classification (substance hazardous to reproductive health). However, there are various studies in the literature that present conflicting and inconsistent findings regarding the toxicological effects of lilial (Jablonská et al., 2023). Lilial is identified as one of the 26 fragrance components that can cause allergic contact dermatitis in cosmetic products (Heisterberg et al., 2011). The European Chemicals Agency's suspicions regarding lilial's potential as an endocrine disruptor remain controversial.

4. Conclusion

There has been a rapid increase in the global use of industrial products, and this rise has led to widespread undesirable effects on pregnant women and fetuses. This article discusses the BMHCA molecule and its derivatives, which were banned in the European Union in 2022 due to their negative effects on the reproductive system and pregnancy. Comparative toxicity studies were conducted using lilial and synonymous compounds obtained from PubChem. In these studies, 2-(4-tert-butylphenyl) propanal, 3-(4-tert-butylphenyl)-2-methylpropanal, and lilial were found to possess moderate to high levels of toxicity. Common characteristics of all these compounds include a high ability to cross the blood-brain barrier (BBB) and a moderate to high potential for carcinogenicity. Due to the conflicting results in the literature and ongoing widespread exposure to this substance in daily life, further experimental studies are needed to clarify the toxicological effects of lilial.

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COMPARISON OF MATERNAL SERUM VITAMIN D LEVELS IN PREECLAMPTIC AND HEALTHY PREGNANT WOMEN

PREEKLAMPTİK VE SAĞLIKLI GEBE KADINLARDA MATERNAL SERUM D VİTAMİNİ DÜZEYLERİNİN KARŞILAŞTIRILMASI

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Abstract
This study aimed to examine the levels of 25 hydroxy vitamin D (25-OH D) in preeclampsia and normotensive pregnant groups. A total of 70 pregnant women who came to the Health Sciences University, Istanbul Gaziosmanpasa Training and Research Hospital for delivery at 35-40 weeks of gestation were included in the study. The study group consisted of 35 preeclamptic (n=35) and 35 normotensive (n=35) pregnant women. Demographic, obstetric, and laboratory results of the two groups were compared in terms of 25-OH D levels. There was no statistically significant difference with the control group in terms of maternal age, gestational age at birth, gravidity, parity, number of abortions and living children, and BMI (p>0.05). When the mean diastolic blood pressure and systolic blood pressure of the groups were compared, the mean blood pressure of the individuals in the preeclamptic group was found to be statistically and significantly higher than the mean systolic and diastolic blood pressure of the individuals in the control group (p<0.05). When the groups were compared in terms of LFT (ALT, AST), proteinuria in urine, and platelet levels in blood, no statistically significant difference was found between the groups. Vitamin D levels were found to be statistically significantly lower in preeclamptic pregnancies compared to normotensive pregnancies. Low maternal vitamin D levels may play a role in the etiology of essential hypertension and preeclampsia, and more comprehensive research is needed on the potential positive effects of additional vitamin D supplementation.
Keywords: Complications, Hypertension, Preeclampsia, Pregnancy, Vitamin D.

Öz
Bu çalışma, preeklamptik ve normotansif gebelerde 25-hidroksi vitamin D (25-OH D) düzeylerini incelemeyi amaçlamaktadır. Çalışmaya, Sağlık Bilimleri Üniversitesi İstanbul Gaziosmanpaşa Eğitim ve Araştırma Hastanesi'nde 35–40. gebelik haftalarında doğum yapmak üzere başvuran toplam 70 gebe kadın dahil edilmiştir. Çalışma grubu, 35 preeklamptik (n=35) ve 35 normotansif (n=35) gebeden oluşmaktadır. Her iki grubun demografik, obstetrik ve laboratuvar verileri 25-OH D düzeyleri açısından karşılaştırılmıştır. Anne yaşı, doğumdaki gebelik haftası, gravide, parite, düşük ve yaşayan çocuk sayısı ile beden kitle indeksi açısından kontrol grubu ile preeklamptik grup arasında istatistiksel olarak anlamlı bir fark bulunmamıştır (p>0.05). Grupların ortalama diyastolik ve sistolik kan basıncı karşılaştırıldığında, preeklamptik gruptaki bireylerin ortalama kan basıncı değerleri, kontrol grubundakilere kıyasla istatistiksel olarak anlamlı düzeyde daha yüksek bulunmuştur (p<0.05). ALT, AST gibi karaciğer fonksiyon testleri, idrarda proteinüri ve kandaki trombosit düzeyleri açısından gruplar arasında anlamlı bir fark saptanmamıştır. Ancak, vitamin D düzeyleri preeklamptik gebelerde normotansif gebelere kıyasla istatistiksel olarak anlamlı şekilde daha düşük bulunmuştur. Düşük

maternal vitamin D düzeylerinin esansiyel hipertansiyon ve preeklampsi etiyolojisinde rol oynayabileceği düşünülmektedir. Bu nedenle, ek vitamin D takviyesinin potansiyel olumlu etkilerini değerlendirmek üzere daha kapsamlı çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Komplikasyonlar, Hipertansiyon, Preeklampsi, Gebelik, Vitamin D.

1. Introduction

Preeclampsia is one of the leading causes of maternal and fetal mortality and morbidity, despite advances in the field of obstetrics. Although the exact cause of preeclampsia is unknown, it complicates 5-8% of nulliparous women and 2-3% of all pregnancies (Sibai et al., 2005). Hypertension during pregnancy can manifest as gestational hypertension, preeclampsia, or eclampsia. Numerous markers that could be significant in predicting preeclampsia have been investigated, and the changes in maternal 25-hydroxyvitamin D levels throughout the trimesters of pregnancy have been evaluated in many studies (Baker et al., 2010). During pregnancy and lactation, changes occur in most vitamins and minerals. Deficiencies in some of these vitamins and minerals are thought to be responsible for a range of conditions, from maternal bone mineral loss to preeclampsia.

Vitamin D plays a crucial role in preventing calcium malabsorption and mitigating bone loss. Upon reviewing clinical studies concerning the relationship between Vitamin D and pregnancy outcomes, it has been suggested that Vitamin D deficiency may be associated with an elevated risk of preeclampsia, gestational diabetes, low birth weight, preterm birth, cesarean delivery, and infectious diseases. However, it has been emphasized that further randomized controlled trials are necessary to substantiate these associations. Recent studies have demonstrated a link between Vitamin D and hypertension. The renin-angiotensin system is well recognized for its significant role in the regulation of blood pressure. It has been established that Vitamin D deficiency directly influences the renin-angiotensin system, consequently increasing the risk of hypertension (Urrutia & Thorp, 2012). 25-OH D possesses essential roles in implantation and placentation, in addition to its angiogenic and anti-inflammatory properties. Certain studies have indicated that elevated levels of 25-OH D may offer protection against preeclampsia. In this context, it has been proposed that severe 25-OH D deficiency, characterized by levels below 10 ng/ml, may heighten the risk of preeclampsia and eclampsia during pregnancy (Rammos et al., 2008).

Recommendations regarding screening and mineral and vitamin supplementation in pregnant women remain a subject of debate. The aim of this study was to investigate the relationship between 25-OH D levels in preeclamptic and normotensive pregnant women.

2. Material and Methods

This study was conducted following decision number 67, dated 08/06/2022, by the Local Ethics Committee of the University of Health Sciences, Istanbul Gaziosmanpasa Training and Research Hospital. Each patient participating in the study was informed, and only those who provided informed consent by signing the participation form were included in the study.

The study was carried out with 70 pregnant women who presented to the Gynecology and Obstetrics Clinic of Gaziosmanpasa Training and Research Hospital for pregnancy follow-up. Pregnant women who had regularly used 25-OH D during pregnancy were excluded from the study. Based on their diagnosis, the participants were divided into two groups: normotensive pregnant women (n=35) (Group 1) and preeclamptic pregnant women (n=35) (Group 2). The demographic and laboratory results, as well as 25-OH D levels, were compared between the two groups.

The diagnosis of preeclampsia was made according to the criteria outlined in the American College of Obstetricians and Gynecologists (ACOG-2013) guidelines on hypertension during pregnancy. Preeclampsia is defined as a condition in which arterial blood pressure is equal to or exceeds 140 mmHg systolic and/or 90 mmHg diastolic in two separate measurements taken at least four hours apart, occurring after the 20th week of pregnancy, in conjunction with proteinuria—measured as 300 mg/dl or more per 24-hour urine collection, or a dipstick reading of 1+ or higher (8). The normotensive group included pregnant women whose blood pressure was normal before pregnancy (systolic/diastolic <120/80 mmHg). Pregnant women diagnosed with essential hypertension, characterized by elevated blood pressure prior to pregnancy (systolic >140 mmHg and diastolic >90 mmHg), were categorized into the essential hypertensive group.

The study cohort comprised pregnant women aged 18 to 40 years who presented to the Department of Obstetrics and Gynecology between 35 and 40 weeks of gestation. Participants with a history of metabolic diseases, thyroid disorders, uterine anomalies, multiple pregnancies, pregnancies achieved through assisted reproductive techniques, known genetic and structural anomalies in fetuses, intrauterine growth restriction, pregestational and gestational diabetes, membrane rupture, chorioamnionitis, fetal tachycardia, or unexplained fever were excluded from the study.

The control group consisted of healthy pregnant women who delivered between the 35th and 40th weeks of gestation without any medical conditions or

adverse obstetric features (such as a history of diabetes, hypertension, obesity, or thyroid disorders). Clinical parameters assessed included age, body mass index, gestational age, gravidity, history of abortion, neonatal weight, and Apgar scores at 1 and 5 minutes.

2.1. Laboratory analysis

In the literature, various threshold values such as 15, 20, and 30 ng/ml have been observed for the diagnosis of 25-OH D deficiency. Based on a series of biomarkers, a minimum serum concentration of 20 ng/ml has been considered desirable. Consequently, deficiency is now defined as a serum level of 20 ng/ml, which has also been utilized as the cutoff value in this study (Holick et al., 2011).

2.2. Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 15.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were expressed as standard deviations and means for numerical variables. The Kolmogorov-Smirnov test was employed to assess the normal distribution of variables, while the Mann-Whitney U test was used for subgroup comparisons. For categorical variables, the Pearson Chi-Square test was applied. A p-value of <0.05 was considered statistically significant.

3. Results and/or Discussion

Comparisons of sociodemographic and clinical data between the groups are presented in Table 1. No significant differences were observed for age, body mass index, gravidity, parity, number of abortions, gestational week, birth weight, and Apgar scores when compared to the control group (p>0.05). The comparison of laboratory values between the groups is displayed in Table 2.

Table 1. Comparison of Sociodemographic and Clinical Data Between Groups

Variables	Normotensive n=35	Preeclampsia n=35	*P*
Age (years)	29.17 ± 6.75	28.82 ± 7.53	0.842
BMI (kg/m^2)	27.89± 4.05	27.02 ± 4.16	0.337
Gravidity (n)	2.77 ± 1.73	2.82 ± 2.09	0.7
Parity (n)	2.00 ± 1.23	2.02 ± 1.33	0.921
Abortus (n)	0.77 ± 1.03	0.74 ± 1.06	0.825
Gestational Week (week)	36.62 ± 2.46	36.51 ± 2.57	0.902
Birth Weight (g)	2982.71 ± 624.56	2911 ± 714.84	0.778
APGAR score	13.00 ± 5.63	14.05 ± 8.37	0.713

BMI: Body mass index, kg: kilogram, g: gram, p<0.005 was considered statistically significant

Table 2. Comparison of laboratory values of groups

Variables	Normotensive n=35	Preeclampsia n=35	*P*
AST(IU/L)	28.49±46.2	33.97 ± 37.26	0.704
ALT(IU/L)	26.2±51	27.6 ± 34.61	0.094
Platelet (10^3 IU/L)	198.45±56.54	193.11 ± 67.11	0.315
Proteinuria median (min-max)	1 (0-3)	2 (0-4)	0.241
25-OH D(ng/mL)	14.69 ± 9.10	10.2 ± 7.82	0.009

AST: Aspartate Aminotransferase, ALT: Alanine aminotransferase, 25-OH D: 25-hydroxyvitamin D

While Vitamin D levels were significantly lower in the groups (p<0.05), NLO (non-lactate oxidase) values were higher in the preeclamptic and preterm groups compared to the control group (p<0.05). When comparing the average systolic blood pressure between the groups, the average blood pressure of individuals in the preeclamptic group was found to be statistically significantly higher than that of individuals in the other group. Similarly, when comparing the average diastolic blood pressure, individuals in the preeclamptic group exhibited a statistically significantly higher average diastolic blood pressure than those in the other group.

When the groups were compared in terms of LFT (ALT, AST), blood platelets, and proteinuria in urine, no statistically significant difference was found between the groups. When compared in terms of 25-OH D, a statistically significant difference was found between the groups.

Preeclampsia remains one of the leading causes of maternal and fetal morbidity and mortality, despite advancements in the field of obstetrics. Numerous studies are being conducted to enable the early

detection of the disease, based on various events believed to be involved in the pathophysiology of preeclampsia. Among these, measurements of serum calcium, phosphorus, and 25-hydroxyvitamin D levels are included. During pregnancy, 25-OH D deficiency, as well as calcium and phosphorus deficiency, are significant concerning fetal and maternal outcomes. One study investigating the relationship between 25-OH D deficiency and placentation has demonstrated that 25-OH D plays a critical role in the development of vascular pathologies (Novakovic et al., 2009). In a study conducted in Sweden by Linnea Bärebring et al., published in 2016, which examined the relationship between preeclampsia, blood pressure changes, and 25-OH D levels in 1,834 pregnant women, preeclampsia developed in 80 of the women who underwent blood pressure monitoring throughout all three trimesters. In this study, the 25-OH D levels of the women in the first trimester were not associated with preeclampsia. However, a negative correlation was observed between preeclampsia and 25-OH D concentrations in the third trimester. Thus, it was concluded that vitamin D levels in early pregnancy may not play a significant role in placental development and consequently the onset of preeclampsia; however, an increase of at least 30 nmol/L in 25-OH D levels during pregnancy could potentially prevent the development of preeclampsia (Bärebring et al., 2016). Haugen et al., based on recent data indicating low vitamin D levels in preeclamptic nulliparous women, found that pregnant women with dietary vitamin D intake levels of 15-20 µg/day had a 27% lower incidence of preeclampsia compared to those receiving lower doses of vitamin D (Haugen et al., 2009). Additionally, a study conducted by Andersen et al. noted that increased plasma concentrations of 25-OH D were associated with a reduced risk of hypertension (Andersen et al., 2015).

It has been stated that placental dysfunction or insufficiency, abnormal angiogenesis, and systemic inflammation, along with hypertension, may contribute to an increased risk of preeclampsia through biological and molecular pathways involving vitamin D (Andersen et al., 2015; Wei et al., 2012). In a study comparing women with preeclampsia to those without, it was found that levels of 25(OH) D vitamin were lower in cases of preeclampsia. Consequently, it has been suggested that vitamin D supplementation in the early stages of pregnancy may help prevent the risk of preeclampsia (Bodnar et al., 2007). A high prevalence of preeclampsia has been observed among women with 25-OH D deficiency, and studies have demonstrated that 25-OH D supplementation during pregnancy results in a reduction in the incidence of preeclampsia (Andersen et al., 2015; Baker et al., 2010; Bodnar et al., 2007; Haugen et al., 2009; Pedersen et al., 1984; Wei et al., 2012). In our study, a significant relationship was found between 25-OH D levels and preeclampsia. The prevalence of deficiency and insufficiency of 25-OH D was significantly higher in preeclamptic women compared to healthy pregnant women, and serum levels of 25-OH D were significantly lower.

Our study demonstrated a high prevalence of 25-OH D deficiency among pregnant women. Considering the entire study population, the frequency of women with adequate 25-OH D levels was only 15.7%. The rates of deficiency and insufficiency were 64.3% and 20%, respectively, which are concerningly high. The causes of deficiency and insufficiency among pregnant women may include inadequate exposure to sunlight, lack of supplementation of foods with 25-OH D, and a deficiency in knowledge and awareness. It is evident that there is a need for awareness projects among healthcare professionals and at the community level regarding this issue within the framework of maternal and child health.

The low serum concentrations among preeclamptic women may indicate the underlying role of 25-OH D deficiency in the pathogenesis of the disease. The women in the control group were of similar age, had similar numbers of pregnancies, births, and miscarriages, were non-smokers, had no history of preeclampsia, were from the same geographic region, and were evaluated in the same time period as preeclamptic women, thus eliminating the effects of many other factors that could have an effect on preeclampsia. This finding aligns with similar studies conducted in various geographical regions worldwide and demonstrates that 25-OH D levels are significantly lower in preeclamptic women compared to healthy pregnant women. Sadin et al. reported a prevalence of 25-OH D deficiency of 60% among preeclamptic women in their study conducted in Iran, whereas the same prevalence in the control group was only 10%. Despite receiving similar amounts of 25-OH D supplementation, they reported that serum 25-OH D concentrations were significantly lower in preeclamptic women (Sadin et al., 2015). Bakacak et al. demonstrated that 25-OH D levels are lower in both preeclamptic and eclamptic patients compared to healthy normotensive pregnant women (Bakacak et al., 2015). Gholami et al. showed that the average serum 25-OH D level in healthy pregnant women was significantly higher than that in women with preeclampsia (Gholami et al., 2022). Richard et al. reported that the median 25-OH D level in preeclamptic women was lower than that in the control group (Richard et al., 2020). They also indicated that women with 25-OH D deficiency (at the threshold of 20 ng/mL) had a higher likelihood of developing preeclampsia (Andersen et al., 2015; Bodnar et al., 2007; Haugen et al., 2009; Wei et al., 2012). Although there are variations among studies

regarding design, the diagnosis of 25-OH D deficiency/insufficiency, timing of sample collection for 25-OH D, and criteria for diagnosing preeclampsia, all these findings point to a relationship between 25-OH D and preeclampsia. This study further confirms this relationship among women in our country.

Baker et al. demonstrated a correlation between midgestational levels of 25-OH D and the severity of preeclampsia, indicating that as 25-OH D levels decreased, the severity of preeclampsia increased (Baker et al., 2010). Additionally, levels of parathyroid hormone, calcitonin, and 1-25 dihydroxyvitamin D3 did not show significant changes in patients with preeclampsia compared to those with normal pregnancies (Pedersen et al., 1984). In another study, no significant relationship was found between maternal serum calcium and 25-OH D levels during the first trimester and the development of preeclampsia (Perçin & Kurtoğlu, 2011). Similarly, Bodnar et al. reported that serum 25-OH D levels in preeclamptic women during early pregnancy were lower compared to the control group, noting that a decrease of 5 nmol/L doubled the risk of preeclampsia (Bodnar et al., 2007). However, in our study, no significant relationship was detected between 25-OH D levels and the development of preeclampsia when comparing normotensive pregnant women.

Another aspect to consider in the relationship between vitamin D and preeclampsia is whether there is a correlation between the severity of the disease and 25-OH D levels. Singla et al. reported that all women with prodromal symptoms of eclampsia or who developed eclampsia had 25-OH D levels of less than 20 ng/mL. However, due to the predominance of 25-OH D deficiency in their study population, drawing definitive conclusions is challenging. The average serum 25-OH D levels did not show significant differences between women with and without eclampsia (Singla et al., 2015). Similarly, in this study, no significant differences in 25-OH D levels or the prevalence of deficiency/insufficiency were observed between women with mild and severe preeclampsia. These findings suggest that while 25-OH D may play a preventive role, once preeclampsia manifests, it may not be effective in altering the disease course or controlling its severity. The course of the disease is determined by various factors, including the individual's biological system, levels of oxidative stress, inflammatory mediators, vascular endothelial damage, and immune responses. Conversely, a study conducted in Turkey reported an average 25-OH D level of 10.99 ± 2.91 in women with mild preeclampsia, indicating significantly higher levels compared to those with severe preeclampsia and HELLP syndrome (Aslan et al., 2022). These varying results underscore the need for larger sample studies focusing on the role of 25-OH D in the severity of preeclampsia.

When the controversial results of studies supporting the relationship between 25-OH D and preeclampsia and reporting no evidence for this relationship are evaluated and when current meta-analyses are taken into account, in the light of our current knowledge and evidence, it is thought that 25-OH D deficiency may be a risk factor for preeclamptic disease and supports the importance of antenatal monitoring and support, especially in pregnant women. To clarify these contentious findings, it is necessary and prudent to conduct multicenter, large-sample national studies within individual populations. Such research could significantly contribute to the development of a national management algorithm.

Our study has some limitations. Due to its single-center design, regional characteristics cannot be overlooked, and the results may not be generalizable. The absence of eclamptic cases and the low number of severe preeclampsia cases limit the ability to conduct a definitive analysis of the relationship between disease severity and 25-OH D levels. On the other hand, being one of the studies conducted among women in our country is important for the national knowledge pool and has facilitated the addition of some regional evidence to the literature.

4. Conclusion

In this study, we investigated how 25-OH D levels varied between preeclamptic and control groups consisting of normotensive individuals. Statistical analyses revealed a significant difference in 25-OH D levels between the groups. In most of the studies conducted to date for the early diagnosis and prevention of preeclampsia, it has been stated that low gestational 25-OH D levels may be significant in predicting the disease. However, further research with larger sample sizes is warranted.

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INVESTIGATION OF THE FREQUENCY OF MICROSATELLITE INSTABILITY FROM PATHOLOGICAL TISSUE SAMPLES OF PATIENTS WITH GASTRIC CANCER AND ITS EFFECT ON DISEASE PROGNOSIS AND TREATMENT

MİDE KANSERİ HASTALARINA AİT PATOLOJİK DOKU ÖRNEKLERİNDE MİKROSATELLİT İNSTABİLİTE SIKLIĞININ ARAŞTIRILMASI VE HASTALIK PROGNOZU İLE TEDAVİ ÜZERİNE ETKİSİ

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Abstract
Gastric cancers continues to be one of the most important health problems in our society with high morbidity and mortality rates. Based on genomic characterization, gastric cancer has recently been defined as a heterogeneous disease consisting of different subtypes, each with unique molecular features and specific clinics. In this study, we tried to investigate the frequency of microsatellite instability in gastric cancers and its effects on the disease. In our study, microsatellite instability results of the patients were seen as microsatellite stable (MSS) in 45 (90%) patients, microsatellite instability high (MSI-H) in 2 (4%) patients and microsatellite instability low (MSI-L) in 3 (6%) patients. It was observed that microsatellite stability was an independent risk factor for mortality. (p=0.035). As a result, it was seen that microsatellite stability was a negative risk factor for mortality in gastric cancer, however, it was concluded that microsatellite instability should be evaluated together with other risk factors that may have an effect on the disease.

Keywords: Gastric cancer, Microsatellite instability, Morbidity, Mortality, Treatment.

Öz
Mide kanserleri hem lokal ileri evrelerde hem de metastatik evrelerde sınırlı tedavi seçeneklerinin olduğu, en agresif seyirli malignitelerden birisidir. Genomik karakterizasyona bağlı olarak, mide kanserleri son zamanlarda her biri kendine özgü moleküler özelliklere ve spesifik kliniklere sahip farklı alt tiplerden oluşan heterojen bir hastalık olarak tanımlanmıştır. Bu çalışmada mide kanserlerinde mikrosatelit instabilitesinin sıklığını ve hastalık üzerindeki etkilerini araştırmaya çalıştık. Çalışmamızda hastaların mikrosatelit instabilitesi sonuçlarına bakıldığında 45 (%90) hastada mikrosatelit stabil (MSS), 2 (%4) hastada mikrosatelit instabilite yüksek (MSI-H), 3 (%6) hastada ise mikrosatelit instabilite düşük (MSI-L) olarak görüldü. Mikrosatelit stabil olmanın mortalite açısından bağımsız bir risk faktörü olduğu görüldü (p=0,035). Sonuç olarak, mikrosatelit stabilitesinin mide kanserinde mortalite açısından negatif bir risk faktörü olduğu görüldü, ancak mikrosatelit instabilitesinin hastalığa etkisi olabilecek diğer risk faktörleriyle birlikte değerlendirilmesi gerektiği sonucuna varıldı.
Anahtar Kelimeler: Mide kanseri, Mikrosatellit instabilite, Morbidite, Mortalite, Tedavi.

1. Introduction
Gastric cancer is one of the most aggressive malignancies with limited treatment options in both locally advanced and metastatic stages (Ratti, 2018).

For this reason, many treatment regimens have been tried and researched over the years. For many years, cisplatin and 5-FU/capecitabine with or without epirubicin have been the standard treatment in the perioperative period for gastric cancer. More recently, the taxane-containing FLOT regimen (Docetaxel, Oxaliplatin, Leucovorin and 5-FU) has been shown to be superior in terms of histological response, relapse-free survival and overall survival (Al-Batran, 2019).
The MSI-H phenotype, which results from inactivation of the DNA missmatch repair (MMR) system due to somatic hypermethylation of the MLH1 gene promoter or germline mutations in MLH1, MSH2, MSH6 and PMS2, has recently received much attention because of the marked immunogenicity of MSI-H cancers and their apparent response to immune checkpoint blockade (Kloor, 2016). It has been observed that the frequency of microsatellite instability is not taken into consideration especially in gastric cancer cases seen in our country. Therefore, in this study, we aimed to evaluate the frequency of microsatellite instability in gastric cancer, its effect together with demographic factors, and its effect on treatment and mortality.

2. Material and Methods

2.1 Working Group

Our study is a prospective cross-sectional study. It was conducted between 01.06.2021-30.09.2022 in patients followed up with the diagnosis of stomach cancer in the Medical Oncology Department of Atatürk University Research Hospital. Patients who underwent MSI examination at the Ataturk University Research Hospital Genetics Laboratory and those who signed an informed consent form were accepted into the study. Patients who did not sign the informed consent form were not accepted into the study. Demographic characteristics of the patients included in the study (age, gender, systemic disease, smoking history, alcohol use history), gastrectomy history, tumor localization, metastasis status, number of treatments and treatment protocols, progression-free survival time, and exitus status were recorded from patient files and the hospital electronic information system.

2.2 MSI Detection

After the patients received pathological diagnosis, MSI study was performed on pathological tissue samples at the Medical Genetics Laboratory of Atatürk University Research Hospital. In this study, specific regions containing microsatellite sequences were amplified as short DNA fragments with the kit used on the EasyPGX® platform, and then stability/instability status was determined for each marker with specific probes after 16 denaturation and hybridization steps. Analysis was performed by comparing individual samples and positive controls for each marker (Table 1).

Table 1. Markers for MSI detection

Marker	Gen	Chromosome
BAT25	cKIT	4 (4q12)
BAT26	MSH2	2 (2p21-p16.3)
NR21	SLC7A8	14 (14q11.2)
NR22	STT3A	11 (11q24.2)
NR24	ZNF2	2 (2q11.1)
NR27	BIRC3	11 (11q22.2)
CAT25	CASP2	7 (7q24)
MONO27	MAP4K3	2 (2p22.1)

2.3 Statistical Analysis

Statistical analyses were performed using SPSS version 15 software. The conformity of variables to normal distribution was examined using visual (histogram and probability plots) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Descriptive analyses were given using median and interquartile range for variables that were not normally distributed and mean ± standard deviation for variables that were normally distributed. Mann Whitney U test was used to evaluate differences between groups in continuous variables. Categorical variables such as diabetes, hypertension, tumor size, tumor location, disease stage, history of gastrectomy, number of treatments received, and microsatellite instability were expressed as numbers and percentages. The presence of differences between groups in terms of these frequencies was compared using Chi-square or Fisher tests, as appropriate. The effects of microsatellite instability and stage on survival were examined using the log rank test. Survival rates were calculated using Kaplan-Meier survival analysis. A separate log rank analysis was used to calculate the effects of microsatellite instability on survival, adjusting for disease stage. Type-1 error levels below 5% were interpreted as statistically significant.

3. Results and Discussion

A total of 50 gastric cancer patients were included in our study. 37 (74%) of the patients were male and 13 (26%) were female, the mean age was 60±10 and the median was 62 (minimum-maximum; 32-82). Demographic characteristics of the patients, smoking, alcohol use and additional systemic disease status such as diabetes mellitus and hypertension are presented in Table 2.
After the patients were diagnosed with pathological gastric cancer, the disease was staged according to

their pathological features and PET-CT images. In the staging, 30 (60%) of the patients were determined to have stage-4 (end-stage) metastatic gastric cancer.

Table 2. Demographics

Age, years, median (minimum–maximum)	62 (32-82)
Female, n (%)	13 (26)
Male, n (%)	37 (74)
Systemic Diseases, n (%)	
Diabetes Mellitus, n (%)	7 (14)
Hypertension, n (%)	8 (16)
Coronary artery disease, n (%)	4 (8)
Atrial fibrillation, n (%)	1 (2)
Chronic obstructive pulmonary disease, n (%)	2 (4)
Smoking, n (%)	
Yes	32 (64)
No	16 (36)
Alcohol, n (%)	
Yes	11 (22)
No	39 (78)

There were no stage 1 patients among the patients. In the distribution of the patients according to the regions where they metastasized in their PET-CTs, 49 (98%) patients were observed to have lymph node metastasis. In terms of the frequency of metastasis, the lymph nodes were the most common site of metastasis. This was followed by the liver in 19 (38%). Other metastasis sites were the peritoneum, lung, adrenal gland, bone, brain, and ovaries.

The follow-up period of the patients was 12±2 months (minimum-maximum; 1 month-22 months). During the follow-up period, progression was observed in 25 patients (50%). It was determined that 20 patients (40%) died. It was observed that the disease progressed in all 20 patients who died. Factors affecting progression and mortality are shown in Tables 3 and 4.

The distribution of 50 gastric cancer patients MSI results was as follows: 45 (90%) were seen as microsatellite stable (MSS). Microsatellite instability high (MSI-H) was determined in 2 (4%) patients and microsatellite instability low (MSI-L) was determined in 3 (6%) patients.

When the relationship between progression status and MSI was evaluated, patients were divided into two groups with MSS and without MSS, and the effect of the two groups on progression was not found to be statistically significant (log Rank value = 0.071). When the same comparison was made in terms of survey, the mortality of the non-MSS group was found to be significantly lower. (log Rank p value = 0.022)

The log Rank test was also performed to evaluate the effect of the disease stage on mortality, and it was seen that mortality increased as the disease stage increased (log Rank p value = 0.033). Thereupon, a new log Rank model was created to understand whether the disease stage changed the effect of MSI stability on mortality, and it was found that the non-MSS group was associated with mortality independently of the disease stage (log Rank p = 0.035).

When we evaluated the current results, no significant relationship was seen between MSI and prognosis. When we look at the relationship between MSI and mortality, it was seen that having MSS was associated with mortality.

As in other studies (Halling, 2019), In our study, low level MSI was observed in gastric cancers, and the current situation was thought to be low due to the small number of samples in our study or because the molecular results of patients with advanced stage disease resulted in a high rate of MSS.

When we investigated the effect of the current MSI status on disease prognosis, it was observed that MSI had no effect on disease prognosis. It has been seen that there are studies that support and do not support this situation (An, 2012; Choi, 2014).

The strength of our study was that there were limited studies on MSI and stomach cancer in our country. It was observed that the studies were generally conducted in Asian societies where stomach cancer is very common. There was no study that could show the level of MSI in stomach cancers in our country. Therefore, our study was a study that could evaluate the relationship between stomach cancer and MSI. In addition, we examined other factors that may have an

effect on morbidity and mortality in gastric cancer and evaluated whether there is a relationship between these factors and MSI.

Table 3. Factors that effect disease progresion

Progression	**No**	**Yes**	***P* value**
Age, years, mean ± standard deviation	59±10	62±10	0.091
Smoking, n (%)	15 (%60)	17 (%68)	0.556
Alcohol, n (%)	7 (%28)	4 (%16)	0.306
Hypertension, n (%)	3 (%12)	5 (%20)	0.702
Progression	**No**	**Yes**	***P* value**
Diabetes mellitus, n (%)	5 (%16)	3 (%12)	1.0
Tumor size, median cm (min-max)	5 (2-12)	5 (3-12)	0.628
Intragastric localization of the tumor, n (%)			
Corpus	9 (%36)	8 (%32)	0.622
Cardia	11 (%44)	9 (%36)	
Antrum	5 (%20)	8 (%32)	
Gastrectomy, n (%)	9 (%36)	7 (%28)	0.544
Disease stage, n (%)			
2	6 (%24)	2 (%8)	0.180
3	7 (%28)	5 (%20)	
4	12 (%48)	18 (%72)	
Number of chemotherapy, n (%)			
1	13 (%52)	6 (%24)	0.044
2	11 (%44)	11 (%44)	
3	1 (%4)	5 (%20)	

Table 4. Factors that effect mortalitiy

Mortality	**Alive**	**Ex**	***P* value**
Age	60±10	61±10	0.751
Smoking, n (%)	20 (%66.7)	12 (%60)	0.630
Alcohol, n (%)	9 (%30)	2 (%10)	0.163
Hypertension, n (%)	4 (%13)	4 (%20)	0.697
Diabetes mellitus, n (%)	5 (%16.7)	2 (%20)	0.687
Tumor size, median cm (min-max)	5 (2-12)	5 (3-12)	0.613
Intragastric localization of the tumor, n (%)			
Corpus	11 (%36.7)	6 (%30)	0.834
Cardia	12 (%40)	8 (%40)	
Antrum	7 (%23.3)	6 (%30)	
Gastrectomy, n (%)	11 (%36.7)	5 (%25)	0.386
Disease stage, n (%)			
2	2 (%23.3)	1 (%5)	0.015
3	10 (%33.3)	2 (%10)	
4	13 (%43.3)	17 (%85)	
Number of chemotherapy, n (%)			0.047
1	14 (&47.7)	5 (%25)	
2	14 (%46.7)	8 (%40)	
3	2 (%6.7)	4 (%20)	

4. Conclusion

MSI is a factor that guides prognosis and treatment, especially in colon cancer, and in our study we wanted to evaluate whether MSI can guide prognosis, mortality and treatment for stomach cancer. We also aimed to see the rates at which MSI is seen in stomach cancers in our center. In this way, we evaluated whether there is a finding that can guide targeted treatments that can be effective in the follow-up and treatment of stomach cancer, which still causes serious mortality in the world and in our country. In addition, studies are needed on this subject with larger populations of patients with stomach cancer. We believe that studying MSI as a genetic marker in patients diagnosed with stomach cancer and investigating the effect of MSI on the prognosis, mortality and treatment of stomach cancer in more detail in future studies will enable the emergence of new modalities that can change the course of the disease and may be useful in the treatment of stomach cancer.

Conflicts of interest: No conflict of interest

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IMMUNOHISTOCHEMICAL EVALUATION, MOLECULAR INVESTIGATION AND DIFFERENTIAL DIAGNOSIS OF A NOVEL ADIPOCYTOKINE CHEMERİN IN OVARIAN AND UTERINE CANCERS

YENİ BİR ADİPOSİTOKİN OLAN KİMERİNİN OVER VE UTERUS KANSERLERİNDE İMMÜNOHİSTOKİMYASAL DEĞERLENDİRMESİ, MOLEKÜLER ARAŞTIRMASI VE AYIRICI TANISI

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Abstract
Patients diagnosed with the following histological types of carcinoma were included in the study: ovarian serous carcinoma (n=16), ovarian endometrioid carcinoma (n=12), ovarian mucinous carcinoma (n=11), uterine serous carcinoma (n=12) and uterine endometrioid carcinoma (n=15). The study was conducted between 2014 and 2023. The study is based on the comparison of two groups of patients with differing levels of organ involvement. Following the extraction of serum from blood samples, the samples were stored in a temperature of -20°C. The measurement of serum chemerin levels was conducted by means of the ELISA method. In the immunohistochemical study, the sections obtained from the tissues in paraffin blocks were stained for chimerine with the Leica Bond max closed system automatic staining device and Bond polymer refine detection (DS9800, Buffalo Grove, United States) DAB compatible kit. In the immunohistochemical study, the presence or absence of chimerin expression in tumour cells was examined. In the group where such expression was observed, the percentage of staining in the cells was calculated. Furthermore, clinical, laboratory and radiological findings were obtained from the hospital system.

Keywords: Adipocyte, Chemerin, Gynecological Carcinoma, Ovarian Carcinoma, Uterine Carcinoma.

Öz
2014-2023 yılları arasında over seröz karsinom (n:16), over endometrioid karsinom (n:12), over müsinöz karsinom (n:11), uterus seröz karsinom (n:12) ve uterus endometrioid karsinom (n:15) tanılı olgular çalışmaya alınmıştır. Çalışma iki farklı organ tutulumu olan hasta gruplarının birbiriyle karşılaşılması üzerine kuruludur. Kanlardan serum elde edildikten sonra -20°C'de serumlar saklanıp, serum chemerin düzeyleri ELISA yöntemi ile ölçülmüştür. İmmünohistokimyasal çalışmada, parafin bloklardaki dokulardan elde edilen kesitlerde Leica bond max kapalı sistem otomatik boyama cihazı ile Bond polymer refine detection (DS9800, Buffalo Grove, United States) DAB uyumlu kit eşliğinde chemerin çalışılmıştır. İmmünohistokimyasal çalışmada tümör hücrelerinde chemerin ekspresyonunun varlığı/yokluğu incelenmiştir. Ekspresyon izlenen grupta hücrelerin yüzde kaçında boyanma olduğu hesaplanmıştır. Hastane sisteminden hastalara ait klinik, laboratuvar ve radyolojik bulguları da verifiye edilmiştir.

Anahtar Kelimeler: Adiposit, Kemerin, Jinekolojik Karsinom, Over Karsinomu, Uterin Karsinomu.

1. Introduction

Uterine cancer is the most prevalent malignancy in the field of gynaecology, with a high mortality rate. Current global estimates place uterine cancer as the fourth most prevalent cancer among the female population worldwide (Teng et al., 2023). A correct diagnosis is paramount for the prognosis and appropriate treatment of patients with this condition. Early diagnosis can facilitate timely treatment decisions and reduce the economic burden of treatment. Ovarian cancer, the third most prevalent cancer within the female reproductive system, is characterised by the highest mortality rate. Ovarian cancer is characterised by its biological behaviour and the absence of specific early symptoms, which often results in diagnosis at an advanced stage of the disease. Approximately 60-70% of cases are diagnosed at FIGO III and IV stages (Maringe et al., 2012). This late diagnosis inevitably results in a poorer prognosis for patients. While the five-year survival rate for patients with stage I and II disease is 80-90%, this rate drops to 30-50% for stage III and IV patients. Despite advances in our understanding of the biology of this cancer, there is still a lack of markers with high specificity and sensitivity for ovarian cancer that have entered medical practice. While the most widely used biomarker of ovarian cancer is plasma CA-125 levels, this is not applicable as a screening test (87% specificity, 70% sensitivity) (Stewart et al., 2019). The identification of novel biomarkers for the diagnosis and prevention of ovarian cancer is, therefore, a significant area of research (Menon et al., 2021).

White adipose tissue is an active organ that secretes a variety of proteins, known as adipocytokines, which play a role in the regulation of metabolism, immunity, the endocrine system and inflammation (Juge-Aubr et al., 2005). Chemerin, a recently discovered adipokine, has been shown to play a significant role in the process of adipogenesis and the chemotaxis of the innate immune system (Rourke et al., 2013). It has been identified in various tissues, including those found in the adipose tissue, liver, pancreas, and skin, which regulate the function of innate immune cells (Barnea et al., 2008). Chemerin, a recently identified adipokine, has been demonstrated to contribute to adipogenesis and lipid metabolism, cell proliferation, inflammation, and endothelial angiogenesis (Roh et al., 2007). In addition, studies suggest a potential association between chemerin and cancer development. Indeed, studies have shown that chemerin expression is significantly reduced in liver cancer, skin cancer and melanoma compared to normal and/or benign tumours (Pachynski et al., 2012). Conversely, another study found that chemerin expression was elevated in colorectal cancer and gastric cancer (Ahn et al., 2016). These findings suggest that changes in chemerin expression may have a significant effect on tumour formation and progression. However, the role of 'Chemerin' adipocytokinin, categorised as 'new adipocytokines', in ovarian and uterine cancer remains to be fully elucidated. The role of this adipocytokinin in differential diagnosis remains to be elucidated. To the best of the present author's knowledge, no experimental study on this subject has been published in the literature.

The characterisation of the molecular mechanisms involved in cancer progression may facilitate the identification of prognostic markers and new therapeutic targets. The present study aims to contribute to the existing literature by identifying novel adipocytokines involved in ovarian and uterine cancer, by identifying markers that can be used in the diagnosis of ovarian and uterine cancer, and by developing targeted therapy and analysing its usefulness in differential diagnosis.

2. Material and Methods

2.1. Creation of patient and control groups and obtaining serum

Serum samples were collected from patients diagnosed with the following histological subtypes of ovarian carcinoma: serous carcinoma (n = 16), endometrioid carcinoma (n = 12), mucinous carcinoma (n = 11), uterine serous carcinoma (n = 12) and uterine endometrioid carcinoma (n = 15). These samples were collected from the Department of Pathology at Amasya University between 2014 and 2023. Serum samples from a control group (n = 20) were also collected for comparison. Serum samples were then stored at -20°C until ELISA experiments were performed.

2.2. Measurement of chemerin levels in serum by ELISA

The measurement of serum chemerin levels was conducted in accordance with the quantitative sandwich enzyme immunoassay technique, employing an ELISA kit (Elabscience, Houston, Texas, United States of America, Catalogue Number

E-EL-H0698). This commercial kit has a sensitivity of 0.10 ng/mL and both inter- and intra-assay coefficients of variation were <10%. It is imperative to note that all standards and samples were run in duplicate. A volume of 100 µl of each standard and sample was added to the wells. The plate is then covered with gelatin using adhesive tape and incubated at 37°C for 90 minutes. The next step involves the removal of the contents from the wells and the addition of 100 µl of biotinylated chemeric antibody at a concentration of 1X. The microplate is then left to incubate at 37°C for a period of 60 minutes. The wells are then emptied and washed on three occasions. Finally, 100 µl of HRP conjugate at 1X concentration is added to each well and the plate is left to incubate at 37°C for 30 minutes. The wells are then washed on five separate occasions. Subsequently, 90 µl of substrate solution is added to the wells, after which the plate is covered and incubated at 37°C for 15 minutes. Immediately after the addition of 50 µl of reaction stop solution to each well, the absorbances of the wells are measured at 450 nm. The concentration and light absorption of the standards are then utilised to calculate the level of chimerine in the serum, employing the standard curve.

2.3. The presence of immunohistochemical staining of chemerin

An immunohistochemical study was conducted in the pathology department of our hospital on paraffin-blocked tissues from patients with newly diagnosed cases. The aim of the study was to examine the presence/absence of expression of tumour cells with immunohistochemical markers.

2.4 Statistical analysis

The data are presented as the mean ± standard error of the mean, and the mean values between the two groups are compared by Student's t test. Pearson's correlation test is utilised to ascertain the relationship between serum and gene expression levels of adipocytokines and other variables. It is imperative to note that all reported confidence interval values are calculated at the 95 per cent level. A P value less than 0.05 is considered to indicate a statistically significant result.

3. Results and Discussion

The results of the study revealed that a total of 39 ovarian cancer groups were diagnosed with ovarian serous carcinoma (OSC) (n:16), ovarian endometrioid carcinoma (OEC) (n:12), and ovarian mucinous carcinoma (OMC) (n:11). In addition, 27 uterine cancer groups were diagnosed with uterine serous carcinoma (USC) (n:12), uterine endometrioid carcinoma (UEC) (n: 15) diagnosed with a total of 27 uterine cancers compared with the control group (CG) (n=20), it was found that serum chemerin levels did not show a statistically significant change in both ovarian and uterine cancer groups compared to the control group (Table1-3).

The ovarian cancer group exhibited a p-value of 0.52 when compared to the control group, and the uterine cancer group demonstrated a p-value of 0.35 when contrasted with the control group.

Table 1. Pathological data of carcinomas

Parameters	OSC	OEC	OMC	USC	UEC
Tumor size	(n)	(n)	(n)	(n)	(n)
T1	12	11	11	10	12
T2	4	1	0	2	3
T3	0	0	0	0	0
Nodal statü	(n)	(n)	(n)	(n)	(n)
N0	12	10	10	4	6
N1	4	2	2	4	4
N2	0	0	0	4	5
FİGO stage	(n)	(n)	(n)	(n)	(n)
I	12	11	11	10	12
II	4	1	0	2	3
III	0	0	0	0	0

Table 2. Immunohistochemical data

Estrogen receptor	**(n)**	**(n)**	**(n)**	**(n)**	**(n)**
Negative	**11**	**4**	**6**	**4**	**0**
Positive	**5**	**8**	**5**	**8**	**15**
P53	(n)	(n)	(n)	(n)	(n)
Mutant	16	1	0	12	0
Not mutant	0	11	11	0	15
WT1	(n)	(n)	(n)	(n)	(n)
Negative	1	11	11	3	10
Positive	15	1	0	9	5
Chemerin	(n)	(n)	(n)	(n)	(n)
Negative	14	11	11	12	14
Positive	2	1	0	0	1

Table 3. Serum data and blood pressure data

Serum chemerin (ng/ml)	371.05±84.35	363.27±77.49	373.24±61.49	348.27±57.46	375.82±64.44
Total cholesterol (mg/dl)	168.18±41.60	153.2± 40.64	128.18±21.60	168.26±41.25	148.36±45.25
Triglycerides (mg/dl)	146.28±52.31	136.22±45,26	166.36±56.21	156.28±52.26	146.28±54,32
HDL cholesterol (mg/dl)	37.68 ± 12.66	36.67± 10.61	40.68± 13.60	38.95± 11.26	36.69± 13.46
LDL cholesterol (mg/dl)	101.12±35.33	101.25±35.30	100.24±31.30	104.12±34.23	103.03±36.12
Systolic blood pressure (mmHg)	129.88±12.22	129.88±12.22	129.88±12.22	129.88±12.22	129.88±12.22
Diastolic blood pressure (mmHg)	81.63± 11.00	80.62± 10.00	82.63± 10.23	81.98± 12.00	80.63± 10.10

Among gynaecological malignancies, ovarian cancers have the highest mortality rate. Ovarian serous carcinoma accounts for 46% of surface epithelial tumours of the ovary. Mucinous carcinomas, on the other hand, are observed less frequently. Despite the observed variations in ovarian cancer incidence across different geographical regions, the mortality rate stands at 9%, with a five-year survival rate of approximately 41.0% (Nucci et al., 2009). The majority of endometrial carcinomas are of the endometrioid type, while mucinous and endometrial serous carcinomas are less common (Tavassoli et al., 2003). However, endometrial serous carcinomas are responsible for approximately 40% of endometrial cancer-related deaths (Moore et al., 2011). Despite the utilisation of analogous chemotherapeutic agents in tumours originating from both organs, treatment algorithms diverge (Zhang et al., 2020). Furthermore, the differing staging of primary ovarian and primary endometrial tumours underscores the necessity for a differential diagnosis of these tumours (Tavassoli et al., 2003). White adipose tissue is an active organ that secretes a variety of proteins, known as adipocytokines, which play a role in the regulation of metabolism, immunity, the endocrine system and inflammation. Chemerin, a recently discovered adipokine, has been shown to play a significant role in the process of adipogenesis and the chemotaxis of the innate immune system. The role of 'Chemerin' adipocytokinin, which is currently termed 'new adipocytokines', in ovarian and uterine cancers is not yet fully understood. The potential of this adipocytokine in the context of differential diagnosis remains to be elucidated. To the best of the authors' knowledge, there have been no experimental studies on this subject in the literature. The present study aims to determine the role of this adipocytokinin in the etiology of ovarian and uterine cancers, to analyse its usability in differential diagnosis and to contribute to the existing literature on the subject.

In the present study, no significant difference was observed between the groups with regard to serum chemerin levels. It is well established that there is a close relationship between metabolism and reproductive function (Schneider et al., 2005). Adipose tissue is recognised as an endocrine organ that can influence fertility through the secretion of adipokines, which are cytokines involved in various physiological processes (Scheja et al., 2019). These biologically active proteins are recognised as the principal regulators of whole body energy homeostasis (Luo et al., 2016). A substantial body of research has already identified and discussed the important roles of leptin and adiponectin in different physiological processes, including reproduction (Barbe et al., 2019). In this study, the focus has been directed towards the effect of Chemerin adipocytokinin on reproductive system cancers, which have been identified and recognised as significant regulators of energy metabolism. Chemerin and its primary receptor CMKLR1 are expressed in white adipose tissue, and elevated circulating levels of this adipokine have been observed in obesity and metabolic syndrome (Bozaoglu et al., 2007). Chemerin is a proinflammatory cytokine that recruits and activates immune cells and contributes to inflammation by promoting macrophage adhesion to vascular cell adhesion molecule-1 (VCAM-1) and fibronectin, suggesting that it may also play a role in the relationship between obesity and inflammation (Ouchi et al., 2011). In humans, chemerin has been predominantly detected in white adipose tissues, liver and placenta, and to a lesser extent in brown adipose tissue, lungs, skeletal muscles, kidneys, ovaries and heart. Chemerin is a cytokine that plays a regulatory role in various physiological processes, including immune system regulation, angiogenesis and inflammation (Mattern et al., 2014). In the present study, no significant difference was observed between systolic and diastolic blood pressures, HDL, LDL, total cholesterol levels and chemerin. The relationship between chemerin and cancer remains to be fully elucidated. However, it has been demonstrated that chemerin can promote angiogenesis by inducing matrix metalloproteinase secretion and activity. Consequently, it is hypothesised that elevated levels of chemerin may contribute to the initiation of carcinogenesis and subsequent metastasis. In contrast, chemerin has been demonstrated to recruit natural killer cells, which have been hypothesised to function as a primary component in cell defence, exhibiting tumour suppressor properties (Skrzeczynska-Moncznik et al., 2009). Chemerin expression has been observed in mouse ovaries under normal physiological conditions (Goralski et al., 2007). Furthermore, Chemerin has been detected in non-tumour human myometrial cells and fibrotic cells by means of microarray and real-time quantitative polymerase chain reaction (RT-qPCR) (Zaitseva et al., 2008). Chemerin expression has been monitored in human primary cell cultures obtained from stromal and extravillous trophoblastic cells from pregnant women. It has been hypothesised that Chemerin levels increase during decidualisation, potentially contributing to natural killer (NK) cell accumulation and vascular remodelling during the early stages of pregnancy (Carlino et al., 2012). Chemerin, a chemotactic agent, has been detected in the rat placenta during pregnancy and is also expressed in human placenta. The role of Chemerin in placentation is characterised by its ability to regulate NK cell accumulation and endothelial cell morphogenesis during the early stages of pregnancy. In the context of pre-eclampsia, Chemerin has been found to play a protective role by regulating umbilical cord vessel endothelial cell-derived nitric oxide signalling, and is expressed in the umbilical cord (Wang et al., 2015).

4. Conclusion

The objective of the study was to utilise the parameter as a diagnostic tool to alert clinicians to the potential emergence of new lesions during the initial diagnosis and subsequent follow-up of patients diagnosed with ovarian and uterine cancer. Consequently, it would serve as an alternative to the costly and challenging to access imaging methods employed for follow-up. From this standpoint, the objective was to prevent patients from seeking care at third-step hospitals, as it was a non-invasive procedure that could be readily available in smaller medical facilities. Furthermore, given its non-invasive nature, it is anticipated that this procedure would provide a less traumatic experience for patients, enhancing their comfort levels. However, given that no significant difference was observed between the groups, the chemerin cannot serve this purpose at this time.

With regard to pathological contributions, the determination of tissue markers would facilitate the work of pathologists in differential diagnosis of patients and guide them during primary focus analyses of metastatic masses. However, given that no significant differences were observed between the groups, the chemistry cannot fulfil this role at present. It is hypothesised that the limited number of patients is a contributing factor to the absence of a significant difference between the groups. It is

recommended that the study be repeated with a larger patient cohort to ascertain the significance of the findings.

The ethical dimension of the research

Approval was obtained from the amasya university non-interventional clinical research ethics committee on 03.02.2022 with decision no: 19.

Conflicts of interest: No conflict of interest

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ETIOLOGY AND DIAGNOSIS OF MALE INFERTILITY

ERKEK KISIRLIĞININ ETİYOLOJİSİ VE TANISI

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Review Article
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Abstract
The global incidence of infertility is increasing year by year, and infertility has become a major medical, psychological, and social problem. According to the World Health Organization's report in 2023, 17.5% of the population worldwide had fertility problems. During the last 25 years, sperm quantity and quality have shown a great diminishment. The male factor is responsible for approximately 50% of infertility cases. Several factors, such as sperm morphologic problems, genital tract structural anomalies, drugs, genetics, environmental factors, and endocrine dysregulation, have a strong bearing on reproductive health. The causes and mechanisms leading to male infertility are very important to understand in order to diagnose and conduct the treatment of the condition to help a couple conceive. This review intends to give an overview of male infertility with regard to causes and diagnostic methods.

Keywords: Diagnosis, Hypogonadism, Infertility, Male, Spermatogenesis.

Öz
İnfertilitenin küresel görülme sıklığı her geçen yıl artmaktadır ve infertilite önemli bir tıbbi, psikolojik ve sosyal sorun haline gelmiştir. Dünya Sağlık Örgütü'nün 2023 yılı raporuna göre dünya genelinde nüfusun %17,5'inde doğurganlık sorunu yaşamaktadır. Son 25 yılda sperm miktarı ve kalitesinde büyük bir azalma görülmüştür. Kısırlık vakalarının yaklaşık %50'sinden erkek faktörü sorumludur. Sperm morfolojik sorunları, genital sistem yapısal anormallikleri, ilaçlar, genetik, çevresel faktörler ve endokrin düzensizlikleri gibi çeşitli faktörlerin üreme sağlığı üzerinde güçlü bir etkisi vardır. Erkek kısırlığına yol açan nedenler ve mekanizmaların anlaşılması, bir çiftin hamile kalmasına yardımcı olmak amacıyla durumun teşhis edilmesi ve tedavisinin gerçekleştirilmesi açısından çok önemlidir. Bu derlemenin amacı, erkek kısırlığına nedenleri ve tanı metotları açısından genel bir bakış sunmaktır.

Anahtar Kelimeler: Erkek, Hipogonadizm, İnfertilite, Spermatogenez, Tanı.

1. Introduction

Infertility is defined as the failure of pregnancy despite uninterrupted and unprotected sexual intercourse for more than a year (Poulter et al., 2024). According to the World Health Organization's report in 2023, 17.5% of the population worldwide experienced fertility problems (WHO., 2023). The male factor is responsible for approximately 50% of infertility cases (Rambhatla et al., 2024). Worldwide, in the 15-49-year-old male population, infertility affects approximately 2% of the males (Liang et al., 2025). Infertility in males can be due to a number of factors ranging from hypogonadism to varicocele (Jungwirth et al., 2012; Eisenberg et al., 2023). Despite advances in diagnosis and treatment methods, 30% of cases are idiopathic (Karimian et al., 2021).

The testicles have two main functions: spermatogenesis and steroid hormone secretion (Carreau et al., 2007). Spermatogenesis: It is the process of developing spermium from spermatogonia and begins with puberty (Christin et al., 2022; Zhang et al., 2024). Spermatogenesis occurs as a result of serial mitosis and meiosis of germ cells in the seminiferous tubule epithelium. It is a sensitive and complex process controlled through hormonal feedback and genetic mechanisms (Cohen et al., 2024).

The hypothalamic-pituitary-gonadal axis plays a very important role in the hormonal regulation of spermatogenesis. Gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) are the main hormones of this control mechanism (Esteves & Humaidan, 2025). GnRH triggers the release of LH and FSH. LH induces

the release of testosterone from interstitial Leydig cells. FSH affects Sertoli cells in the seminiferous tubules. Sertoli cells produce the regulators and nutrients necessary to maintain spermatogenesis (Oduwole et al., 2018).

In men with impaired secretion of LH and FSH, the diagnosis is hypogonadotropic hypogonadism, and spermatogenesis is interrupted (Fraietta et al., 2013). There are two types of hypogonadotropic hypogonadism. It may be congenital or acquired. Congenital hypogonadotropic hypogonadism is Kallmann syndrome, and the other is idiopathic hypogonadotropic hypogonadism. Acquired hypogonadotropic hypogonadism can be caused by tumors, traumas, infections, secondary to drug therapy, radiation, alcohol, and systemic diseases (Salenave et al.,2012; Fraietta et al., 2013). The incidence of congenital hypogonadotropic hypogonadism is approximately 1-10:100,000, and approximately 60% of cases are caused by Kallmann syndrome and 40% are idiopathic hypogonadotropic hypogonadism (Bianco et al., 2009).

2. Etiology of Male Infertility

2.1. Varicocele

Varicocele is a pathologic dilatation and twisting of the pampiniform venous plexus in the scrotum caused by reflux of the veins, which is most often on the left side. It is present in 15% of all men and is a major cause of male infertility. Varicocele is a multifactorial condition due to genetic, epigenetic, and environmental causes (Naderi et al.,2024). In patients with varicocele, increased scrotal temperature, oxidative stress, and impaired sperm production are the factors that affect the fertility (Hassanin et al.,2018). Varicocele is a surgically repairable cause of male infertility (Rochdi et al., 2024). Microsurgical low ligation of varicocele is the gold standard for surgical therapy (Shiraishi, 2024). Also, varicocelectomy is another choice in the surgical treatment of varicocele, and these treatments support the spermatozoa count, motility, and morphology (Rochdi et al., 2024).

2.2. Cryptorchidism

Cryptorchidism is known as the congenital absence of one or both testicles in the scrotum. It increases the risk of infertility and testis cancer. The most successful treatments for cryptorchidism are orchidopexy and hCG therapy (Hutson, 2009). Testicular descent is the result of interactions between testosterone and its androgen receptor. Also, insulin-like factor 3 (INSL3) / relaxin family peptide 2 (RXFP2) are essential for the first phase of testicular descent. Mutations in these factors are in the etiology of cryptorchidism (Krausz, 2011).

2.3. Genetic factors

Studies on somatic chromosomes in infertile men have shown that approximately 15% of azoospermic men and 5% of oligozoospermic men have an abnormal karyotype. Also, the patient whose sperm count is less than 10 million has the risk of autosomal structural abnormalities 10 times higher than the general population (Van Assche et al., 1996; Vincent et al., 2002). The most common sex chromosome abnormality in men is Klinefelter syndrome (KS; 47XXY) (Xu et al., 2022). The incidence in male newborns is approximately 1/600 (150 per 100,000 live-born boys) (Chang et al., 2020). KS is characterized by bilateral gynecomastia, small testes, aspermatogenesis, androgen deficiency, decreased testicular volume, azoospermia, and reduced intelligence (Bojesen et al., 2007; Blackburn et al., 2025). The typical endocrine finding in KS is hypergonadotropic hypogonadism, and its treatment is testosterone replacement (Chang et al., 2020).

Another genetic disease that causes spermatogenesis disorder is Y chromosome microdeletions. On the long arm of the Y chromosome, three regions are defined: They are defined as azoospermia factors. These (AZFs) are AZFa, AZFb, and AZFc. Any deletion in the AZF region may cause male infertility (Vogt et al., 1996). The most common deletion type is the AZFc region deletion (~80%), followed by AZFa (0.5–4%), AZFb (1–5%), and AZFbc (1–3%) deletions (Krausz et al., 2014). Epithelial cell membranes have the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which encodes an anion-selective channel. Mutations in CFTR cause cystic fibrosis, an inherited disease. In cystic fibrosis, the epithelium of the genital ducts (the epididymis and vas deferens) is damaged, so nearly all patients are infertile (Coatti et al., 2024; Sapru et al., 2025). Approximately 96% of patients with CF have congenital bilateral absence of vas deferens (CBAVD) and are infertile due to azoospermia (de Souza et al., 2018). However, cystic fibrosis patients can also become fathers by using assisted reproductive technology along with surgical sperm retrieval and intracytoplasmic sperm injection (ICSI) (Sapru et al., 2025).

2.4. Environmental factors and lifestyle factors

Environmental factors such as heavy metals, chemicals, pesticides, and radiation negatively affect male fertility. They damage genital organs and disrupt the hormonal balance. Continuous intake of heavy metals (lead, cadmium, mercury, and arsenic) and metal welding fumes such as carbon disulfide and ozone has been negatively associated with sperm quality (Abilash et al., 2024; Adeogun et al., 2024; Bhardwaj et al., 2024; Dubey et al., 2024; Maher et al., 2024). Pesticides may damage fertility by triggering

congenital anomalies, fetal death, and infertility (Mahmoud et al., 2025). Various pesticides are apoptotic inducers for Sertoli cells (Mansukhani et al., 2024; Moreira et al., 2024). Radiation has two types: ionizing and non-ionizing. Exposure to ionizing radiation has been proven to cause male sterility. In males, chronic exposure to non-ionizing radiation may have a negative impact on sperm characteristics such as count, motility, morphology, and viability (Ebrahimi et al., 2024; Simson et al., 2024; Korhonen et al., 2025).

Stress, immobility, obesity, aging, alcohol, cigarettes, and cannabis are the lifestyle factors. Metabolic syndrome is characterized by hypertension, insulin resistance, increased oxidative stress, and inflammation. Its prevalence is nearly 40% of men in Europe (Salvio et al., 2022). Stress has harmful effects on spermatogenesis, sperm count, motility, and sperm quality. Also, a decrease in testosterone, FSH, and LH levels causes deterioration in testicular histopathology (Ilacqua et al., 2018; Yadav et al., 2025). Long hours of sedentary employment cause visceral obesity and chronically high scrotal temperatures; both have a negative impact on sperm production. Nowadays, it turns out that low-to-moderate intensity exercises are more beneficial for male reproductive health than high-intensity exercises, which have a negative effect on sperm (Hamim et al., 2025). Metabolic syndrome has been reported to be related to increased oxidative stress, negatively influencing the spermatogenesis process, which can lower the semen quality and quantity (Bhattacharya et al., 2024).

There is strong proof that smoking has negative effects on spermatogenesis in both infertile and fertile men, which makes it a risk factor for male reproductive health (Cargnelutti et al., 2023). Smoking has detrimental effects on main sperm parameters and male fertility rates. A man who is planning fatherhood has to quit smoking and improve his lifestyle to not face infertility (Fan et al., 2024). Tobacco smoke contains lead and cadmium, which contribute to lower male fertility via oxidative stress pathways, destroying sperm DNA and reducing sperm production (Fan et al., 2024; Marchlewicz et al., 2007). Smoking, specifically, has been closely established to have a negative impact on male reproductive health, and smokers often need more IVF attempts to achieve conception (Fan et al., 2024). Sedentary life and obesity have both been associated with decreased male fertility (Gaskins et al., 2014; Hamim et al., 2025). With advancing age, oxidative stress increases in sperm cells, and DNA repair ability decreases (Kaltsas et al., 2024).

Wi-Fi and mobile phone usage has negative effects on a male's reproductive system. Exposure to radiofrequency electromagnetic radiation, in particular, can harm the male reproductive system by affecting Leydig cell processes, including the generation of testosterone (Jangid et al., 2024). According to studies on animals, cell phone electromagnetic radiation can have a harmful effect on testicular tissue and sperm characteristics such as morphology, motility, viability, and sperm count (Assefa et al., 2025).

Psychoactive drugs could affect fertility by producing testicular oxidative stress, blocking the neuroendocrine axis, and increasing the circulating levels of proinflammatory cytokines that can trigger germ cell apoptosis and testicular degeneration, thus decreasing the quality of semen (Hamed et al., 2023). Studies have indicated that cannabis has been found to reduce sperm count and concentration, induce sperm morphological defects, reduce sperm motility and viability, and impair capacitation and fertilization (Whan et al., 2006; Banerjee et al., 2011). Methamphetamine inhibits testosterone production and increases germ cell apoptosis in rats (Yamamoto et al., 2002).

Cannabis/marijuana: In the United States in 2021, approximately 45% of young adults ages 19 to 30 report using marijuana (Lee et al., 2022). Many studies have demonstrated that tetrahydrocannabinol (THC) reduces male fertility and promotes gonadal dysfunction, particularly at the testis and sperm levels. As a result, THC affects serum testosterone levels and lowers sperm count, motility, normal morphology, and acrosome reaction (Nahas et al., 2002; Gundersen et al., 2015; Y. Li et al., 2022; Truong et al., 2023). Alcohol harms reproductive health by suppressing the hypothalamic-pituitary-gonadal axis, resulting in infertility in men (Finelli et al., 2021). Chronic alcohol consumption also decreases testosterone and LH levels (Moosazadeh et al., 2024).

2.5. Infectious factors

Genital tract infections account for approximately 15-20% of male infertility cases. Infections can affect various parts of the male reproductive tract, including the testis, epididymis, and male accessory sex glands. Urogenital infections can influence spermatozoa at various stages of development, maturation, and transport (Pellati et al., 2008; Sleha et al., 2013). Sexually transmitted infections may cause obstruction and scar tissue formation in the genital tract (Kumar, 2008; Henkel, 2021; Henkel et al., 2021). Chlamydia trachomatis, Neisseria gonorrhoeae, and Treponema pallidum are the most commonly found bacteria involved in sexually transmitted illnesses that interfere with male fertility. Male infertility is less frequently caused by non-

sexually transmitted epididymo-orchitis, which is usually caused by Escherichia coli (Sleha et al., 2013; Ruggeri et al., 2016). Enterococcus faecalis (EF) disrupts sperm concentration and morphology. The incidence of oligozoospermia and teratozoospermia is higher in EF infection (Mehta et al., 2002). Ureaplasma urealyticum is one of the most common causes of male infertility. It impairs sperm quality. It disrupts the motility, density, and morphology of sperm (Ruggeri et al., 2016). Escherichia coli is the most isolated microorganism in genitourinary infections that causes prostatitis and epididymitis. This bacteria has an agglutination effect on sperm. Also, it impairs the acrosomal function (Kaur et al., 2014., Folliero et al., 2022). Genital tract tuberculosis is characterized by granulomas. Usually, the inflammation that follows infection causes granuloma formation. This granuloma causes scarring and obstruction, which leads to infertility (Kumar, 2008). Viral infections like the human papillomavirus (HPV) cause DNA fragmentation in sperm cells, and lower sperm count, concentration, and viability. Coronavirus causes high fever, oxidative stress and sperm DNA fragmentation (Çetinavci et al., 2021). Other viruses that affect semen quality are hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) (Guo et al., 2024).

3. Diagnosis of Male Infertility

To diagnose male infertility, first, a detailed medical history must be taken to examine the risk factors. Then, a physical examination must be performed to eliminate the anatomical abnormalities. In the next step, semen analysis and imaging methods need to be applied. Anatomical causes can be detected with radiological methods (Jhaveri et al., 2010; Krausz, 2011; Jungwirth et al., 2012; Tournaye et al., 2017).

3.1. Semen analysis

Semen analysis is a simple, inexpensive method that provides valuable information for diagnosis. It provides critical information about sperm concentration, motility, and morphology. The WHO manual, 5th edition, was published in 2010 and revised as the 6th edition in 2021. For this manual, normal semen parameters are semen volume ≥1.5 mL, sperm concentration of ≥15 million/mL, total sperm count ≥39× 10^6/mL, motility of ≥32%, sperm vitality ≥58%, and normal morphology of ≥4% (WHO 2010, WHO 2021).

Table 1. Pathological Semen Quality According to (WHO, 2010; WHO, 2021).

Nomenclature	Evaluation Result
Oligozoospermia	Sperm concentration <15 x 10^6/ml; total sperm number <39 x 10^6/ml
Asthenozoospermia	<32% progressively motile spermatozoa
Teratozoospermia	<4% morphologically normal spermatozoa
Oligo-asteno-teratozoospermia	Disturbance of all three parameters
Azoospermia	No spermatozoa in the ejaculate
Cryptozoospermia	Spermatozoa absent from fresh preparation but observed in a centrifuged pellet
Leucospermia (leucocytospermia)	>1 x 10^6 ml leucocytes in the ejaculate
Aspermia	No ejaculate

Semen analysis may show a decrease in sperm count (oligozoospermia), decreased sperm motility (asthenozoospermia), and structurally abnormal sperm (teratozoospermia). Semen volume and pH may give clues about seminal vesicle pathologies (Jungwirth et al., 2012; WHO, 2021; Song et al., 2025). The diagnosis should be made based on at least 2 semen analyses. Factors that may compromise the reliability of the analysis and need to be considered are that sperm samples must be collected appropriately. It should be kept at body temperature during transportation. Sexual intercourse should be avoided for a period of 2-5 days. Before semen collection, a high fever illness should be questioned. Additionally, the antibiotics and medications used should be questioned (Krausz, 2011).

3.2. Endocrine analysis

One of the other important diagnostic methods is hormonal tests. Required to identify endocrine disorders. Testosterone, FSH, and LH levels must be examined for checking the hypothalamic-pituitary-gonadal axis (Viramgami et al., 2025). Pituitary adenomas cause hyperprolactinemia, which

suppresses gonadotropin-releasing hormone (GnRH) secretion and causes infertility (Haidenberg et al., 2024). Thyroid dysfunctions such as hyperthyroidism and hypothyroidism change semen parameters and reduce fertility (Anelli et al., 2024).

3.3. Radiologic analysis

Imaging of the male genital system plays a very important role when investigating the causes of infertility. Ultrasound is considered the gold standard method for scrotal examination. Scrotal lesions like varicoceles, testicular cancers, and epididymal obstruction may be detected by ultrasound examination (Lotti et al., 2021; Lotti et al., 2024). Color Doppler ultrasonography, contrast-enhanced ultrasonography, and sonography allow doctors to evaluate the size, vascularity, and abnormal appearance of structures within the scrotum. Scrotal ultrasonography is also used to investigate scrotal pain, masses, and trauma (Bertolotto et al., 2018; Huang et al., 2020). Transrectal ultrasound is especially used to examine the distal parts of the genital tract. Allows visualization of the vas deferens, seminal vesicles, prostate, and ejaculatory ducts (Sihag et al., 2018). MR imaging has the ability to provide high-quality and multiplanar images. In this way, it is used especially in examining the pathological conditions of the prostate, seminal vesicles, and ejaculatory ducts (Donkol, 2010).

3.4. Genetic analysis

Genetic tests are not needed to be performed on every azoospermic patient who applies for infertility. It should be evaluated according to the clinical characteristics of each patient. There is no need for genetic testing in a patient whose vasa is palpable during physical examination and also whose testicular volume, semen volume, and FSH levels are normal (Wosnitzer, 2014). In addition, genetic testing is not required for patients who have not had infertility problems in the past, patients who have received chemotherapy or radiotherapy treatment, or patients whose sperm concentration in previous tests is >5 million/mL ejaculate fluid (Meistrich, 2013). Patients who need testing are those with suspicion of congenital obstruction, primary testicular failure with low testicular volume, and high FSH. Also, genetic testing is recommended for the patients with oligospermia or azoospermia to identify chromosomal abnormalities (Wosnitzer, 2014). Karyotype analysis is generally used in Klinefelter syndrome (Wosnitzer, 2014; Hssaini et al., 2024). The Y chromosome is responsible for a wide range of processes, from the development of the testicles to spermatogenesis. There are some testis-specific gene regions on the long arm of the Y chromosome. Most of these genes are located in the "azoospermia factor" AZF region and are the most important target in the study of genetic causes of male infertility (Deng et al., 2023; Osadchuk et al., 2024). In Y chromosome microdeletions, AZFa, AZFb, and AZFc gene regions may be analyzed (Krausz et al., 2017). Also, in cystic fibrosis patients, CFTR gene mutations may be searched for diagnosis of infertility (Li et al., 2024).

4. Conclusion

Male infertility is multifactorial, due to endocrine, genetic, anatomic, infection, environmental, and lifestyle causes. Diagnosis generally requires a thorough evaluation that typically consists of a history, physical examination, and semen analysis. Genetic testing, imaging, and medications could be employed in finding underlying problems. Also, lifestyle, diet, smoking, alcohol, and drugs must be controlled. Male infertility is a global health problem that requires a multidisciplinary approach for effective diagnosis and treatment. Ongoing research, public awareness, and advances in medical technology are essential to improve outcomes for affected individuals and couples. Nowadays, there are rapid developments in medicine. The advancements in diagnostic methodologies and treatments, hormone treatment, surgical techniques, and assisted reproductive technologies have benefited many couples so far. Future research on male infertility may uncover the mechanism of idiopathic infertility, examine the prevention of underlying genetic deterioration, and develop new biomarkers and AI-based algorithms. Additionally, stem cell therapies may be tested, and assisted reproductive technologies may be optimized. Research may also focus on developing cost-effective solutions and expanding healthcare delivery to larger areas. In summary, elucidating the etiology of infertility and diagnosing it, together with therapeutic developments, can help effectively manage male infertility.

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ANABOLIC ANDROGENIC STEROID USE DISORDER: A PUBLIC HEALTH ISSUE

ANABOLİK ANDROJENİK STEROİD KULLANIM BOZUKLUĞU: BİR HALK SAĞLIĞI SORUNU

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Abstract
Androgenic-anabolic steroids (AAS) are synthetic substances derived from the male hormone testosterone. According to the current scientific literature, these substances are widely used, particularly by athletes and bodybuilders, to enhance muscle mass. Beyond their use in sports, AAS are increasingly misused by a broader population seeking muscle growth without an increase in fat mass, making AAS abuse a significant public health concern. Due to the physiological, psychological, and social effects associated with AAS use and its widespread prevalence, healthcare professionals should identify patients who may be at risk and stay informed about scientific studies on the adverse health effects of these substances.
The physiological, psychological, and social consequences of AAS use pose serious risks to users' overall health and have far-reaching effects on society. Sudden cardiac death, which has been linked to the use of performance-enhancing drugs, is the most common medical cause of death among athletes. This study highlights the public health significance of AAS use disorder, examines the reasons behind its widespread use, and explores its effects on health and available treatment options. Additionally, it underscores the importance of requesting AAS analyses, which are not routinely performed in forensic autopsy procedures, particularly in cases of suspicious deaths among athletes.

Keywords: Anabolic Androgenic Steroid, Adverse Effects, Forensic Toxicology, Sudden Cardiac Death, Public Health.

Öz
Androjenik-anabolik steroidler (AAS), erkeklik hormonu olan testosterondan türetilen sentetik maddelerdir. Mevcut bilimsel literatüre göre, bu maddeler özellikle sporcular ve vücut geliştirme yarışmalarına katılanlar tarafından kas kütlesi artırma amacıyla yaygın olarak kullanılmaktadır. AAS'nin spor alanında kullanımı dışında, yağ kütlesinde artış olmadan kas kütle artışı sağlamak üzere daha geniş kitleler tarafından suistimal ediliyor olması, AAS'yi önemli bir halk sağlığı endişesi halinde getirmiştir. AAS kullanımına bağlı gelişen fizyolojik, psikolojik ve sosyal etkiler ile yaygın kullanımı nedeniyle, sağlık profesyonelleri AAS kullanma potansiyeli bulunan hastaları tanımalı ve bu maddelerin olumsuz sağlık etkileri hakkında yapılan bilimsel çalışmaları takip etmelidir.
AAS kullanımının yol açtığı fizyolojik, psikolojik ve sosyal etkiler, kullanıcıların genel sağlığını tehlikeye atmakta ve toplum genelinde geniş çaplı etkilere neden olmaktadır. Performans artırıcı ilaçların kullanımıyla da ilişkilendirilmiş olan ani kardiyak ölüm, sporcularda en yaygın görülen tıbbi ölüm nedenidir
Bu araştırma, AAS kullanım bozukluğunun halk sağlığı açısından önemini vurgulamakta, yaygın kullanım nedenlerine, sağlık üzerindeki etkileri ve tedavi yöntemleri ile özellikle sporcularda görülen şüpheli ölüm vakalarında adli otopsi uygulamaları rutininde yapılmayan AAS analizlerinin talep edilmesinin katkılarına dair bir bakış sunmaktadır.

Anahtar Kelimeler: Anabolik Androjenik Steroid, Yan Etkiler, Adli Toksikoloji, Ani Kardiyak Ölüm, Halk Sağlığı.

1. Introduction

Anabolic-androgenic steroids (AAS) are synthetic compounds that mimic the effects of male hormones such as testosterone. Although primarily used to enhance bodybuilding, muscle mass, and athletic performance, AAS carry significant risks to both physical and psychological health. Beyond the well-documented somatic effects, AAS misuse can provoke psychological and behavioral changes and may lead to dependency, potentially causing irreversible health consequences if left untreated. In a study by Torissi et al. of 33 individuals with a history of AAS misuse or phenotypic features suggestive of AAS use, medical records were available for 24 cases; none had a personal or family history of heart disease before age 50. Additionally, toxicological analyses were negative in four cases (Torrisi et al., 2020).

The cardiovascular system is one of the most affected systems by the side effects of AAS use. The most well-known cardiovascular events include impaired left ventricular function associated with hypertrophic cardiomyopathy, myocytolysis, and fibrosis, as well as arterial thrombosis, pulmonary embolism, and left ventricular hypertrophy (Hernández-Guerra et al., 2019).

The World Health Organization (WHO) has stated that AAS use is not limited to athletes and is increasingly prevalent due to aesthetic concerns. Particularly among young individuals, the pressure to enhance muscle mass and the pursuit of an ideal body image are key factors driving AAS use (Gibbons et al., 2020).

1.1. Prevalence of Anabolic Androgenic Steroid Use

AAS use is not limited to athletes; it has spread to a broader population, particularly due to the perception of aesthetic perfection promoted through social media. Young individuals, in particular, turn to these substances in pursuit of a muscular and fit appearance. According to the U.S. National Institute on Drug Abuse (NIDA), anabolic steroid misuse is most commonly observed among male weightlifters in their 20s and 30s. The Monitoring the Future study commissioned by NIDA reported that in 2024, an estimated 0.6% of 8th-grade students, 0.7% of 10th-grade students, and 1% of 12th-grade students admitted to misusing steroids within the past year. These findings indicate that steroid use remains a concerning issue even among high school students (NIDA, 2024).

A study conducted in Turkey has also shown an increase in the use of AAS (anabolic-androgenic steroids) among university students. Research indicates that young people are turning to AAS to enhance their body image and physical attractiveness (Demirtaş & Yalçın, 2017). Additionally, it is noted that this usage is increasingly normalized through social media, and societal pressures further reinforce this trend.

1.2. Reasons for AAS Use

The primary motivation behind AAS use, particularly among young people, is to enhance physical appearance and attractiveness, as well as to improve athletic performance. The perception that a muscular and strong physique is ideal drives young individuals to use such substances.

Another group where AAS use is prevalent includes those experiencing dysmorphophobia, a condition defined as an excessive mental preoccupation with one's physical appearance, regardless of whether there is an actual physical flaw. Individuals with body dysmorphic disorder (BDD) constantly perceive their bodies as flawed in terms of shape, leading them to desire a more muscular and larger physique, which often results in the use of AAS (Gruber & Pope, 2019).

1.3. Negative Effects of AAS Use

While users often expect an increase in muscle mass and improved performance, excessive and uncontrolled use can lead to various health problems. Despite the adverse effects of AAS use, dependency—characterized by chronic AAS consumption—can still occur (Kanayama et al., 2019). According to a model of AAS addiction, the first stage, known as the "myoactive" phase, involves the use of high doses of AAS in combination with a specific diet and intense weight training. The second stage is marked by continued high-dose AAS use, which leads to the development of brain reward mechanisms, thereby contributing to misuse and addiction (Brower, 2002). AAS addiction can severely disrupt users' psychological health by directly affecting brain functions through changes in body chemistry. In most AAS users, low levels of gonadotropins and testosterone have been detected even after discontinuing the substance, demonstrating its negative effects on the reproductive system. Long-term complications of AAS use in women include hirsutism, acne, temporal male-pattern hair loss, voice deepening, and clitoromegaly. Additionally, menstrual irregularities in women may predispose them to long-term cardiovascular diseases, stroke, and other cardiovascular problems (Anawalt, 2019; Bahrke & Yesalis, 2004; Christou et al., 2017).

Cardiovascular risks associated with AAS use include myocardial dysfunction, left ventricular hypertrophy, coronary atherosclerosis, hypertension, life-threatening arrhythmias, and sudden death. Additionally, long-term use leads to increased LDL levels and decreased HDL levels, further elevating the risk of cardiac adverse events (Baggish et al., 2017; Christoffersen et al., 2019; Vanberg & Atar, 2010).

AAS use can precipitate psychiatric and behavioral disorders, manifesting as depression, anxiety, anger outbursts (so-called “steroid rage”), and even psychosis. In an observational study by Christoffersen et al. individuals who misused AAS showed elevated

aggression and violent behavior, as well as a ninefold greater risk of criminal activity and subsequent imprisonment (Christoffersen et al., 2019).

1.4. Prevention and Treatment Approaches

Treating AAS use disorder requires a multidisciplinary approach. According to available evidence, the most effective treatment methods include discontinuing AAS use, managing withdrawal symptoms, combining cognitive and behavioral therapies, and providing symptomatic treatments. In particular, psychological support for individuals can help change misconceptions about body image and assist in combating AAS dependency. Organizing educational programs to explain the potential harms of AAS use may encourage individuals to avoid these substances. Given that young people, among whom AAS use is prevalent, also have high social media usage, awareness campaigns conducted through social media platforms could be an effective strategy to prevent AAS use.

2. Conclusion

Anabolic-androgenic steroid (AAS) use disorder represents a significant public health concern, affecting not only athletes but also the general population—particularly young people—due to its rising prevalence among youth. In addition to the physical and psychological health risks associated with AAS use, the potential for dependency highlights the critical need to raise awareness, provide education and guidance, prevent widespread use, and facilitate access to treatment through effective health policies and public health strategies. Furthermore, in forensic medicine practices, routine AAS analyses are not currently performed in cases of suspicious death. However, when a physical phenotype suggestive of AAS use is observed, conducting AAS analyses on body fluids, tissues, and hair samples can offer crucial support to macroscopic findings and significantly contribute to determining the cause of death.

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