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REVIEW

Genetic heterogeneity and metabolic reprogramming in breast cancer

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ABSTRACT

Breast cancer is the most prevalent cancer and the leading cause of cancer-related mortality among women. Recent breakthroughs in breast cancer therapeutics have significantly enhanced outcomes for hormone receptor-positive and HER2-negative subtypes. However, the emergence of drug resistance, particularly in triple-negative breast cancer, presents a formidable challenge. The intricate interplay of genetic and metabolic diversity within breast cancer cells is pivotal to its development. By reprogramming metabolic pathways, cancer cells can adapt and thrive, meeting the demands of survival, growth, and invasion. These metabolic shifts also play a key role in the development of resistance to conventional therapies. This review explores the genetic and metabolic complexities of breast cancer, emphasizing the diverse subtypes and their unique profiles. We examine how genetic variations and metabolic alterations contribute to breast cancer development and progression, influencing both treatment efficacy and resistance. By integrating insights into the genetic background and metabolic reprogramming of breast cancer subtypes, this review aims to highlight the genetic variations and metabolic alterations that contribute to the pathogenesis of breast cancer, with a vision of advancing more precise and effective targeted therapies as well as discovering of novel diagnostic and prognostic markers.

Keywords: pathogenesis, breast cancer, genetics, biochemistry, metabolism.

INTRODUCTION

Breast cancer (BC), the most common cancer in women, was the second most common cancer in 2022 and accounted for 22% of all female malignancies. It is the leading cause of cancer-related death in women and is becoming more common [1]. BC is both genetically and molecularly heterogeneous disease with several subgroups that represent a wide variety of tumors with different morphological, biochemical, and clinical characteristics. There are several techniques and criterias to classify BC with different purposes. According to prognostic receptor expressions, BC is currently classified as luminal A, luminal B, HER2-positive, and triple-negative [2]. These BC subtypes show different biological behaviors and responses to therapy, which emphasizes the value of individualized treatments.

Research has shown that there are numerous metabolic and genomic changes in BC and this opens the door for the development of novel treatment approaches. Since current treatments are still insufficient despite the advancements, there is still a need for more effective therapies. The majority of BC cases are sporadic; approximately 5-10% of cases demonstrate hereditary traits [3]. Certain pathogenic variations in genes, such as *TP53, BRCA1, BRCA2, ATM, PALB2, CHEK2, BARD1, RAD51C*, and *RAD51D*, are linked to an increased risk of BC [4]. Furthermore, several low-penetrance

alleles and their various combinations is considered responsible for a significant part of BC susceptibility [5].

The metabolic alterations in BC, including glucose, lipids, and amino acids metabolism, show the adaptability of cancer cells and their ability to survive even in challenging conditions [6]. The Warburg effect highlights the metabolic alterations that, through increased glycolysis and disrupted lipid and amino acid metabolism, support tumor growth and survival [7]. These metabolic shifts are one of the major causes of treatment resistance and being crucial for the growth of tumors [8].

In conclusion, combining metabolic and genetic knowledge is important for developing accurate and effective treatments due to different BC subtypes have distinct metabolic changes [9]. To develop novel and focused treatments, more research is required to elucidate the intricate relationships between genetic alterations and metabolic pathways.

Subtypes of BC

Triple-Negative (TNBC), HER2-enriched, Luminal A, and Luminal B are the commonly identified subtypes of BC [10]. It is necessary to understand these various subtypes in order to create individualized and successful therapies for BC patients.

Luminal A

Luminal A subtype is defined by the presence of estrogen receptor (ER) and/or progesterone receptor (PR) and the lack of human epidermal growth factor receptor 2 (HER2). This subtype has a less than 15% expression of Ki-67. Luminal A carcinomas are low grade, slow growing, and have the best prognosis with less incidence of relapse and a higher survival rate [2, 11, 12]. These cancers show a poor response to chemotherapy and a high response to hormone therapy [13].

Luminal B

Compared to luminal A, luminal B tumors are of a higher grade, grow more quickly, and have a worse prognosis. They are characterized by being ER-positive, can be PR-negative, overexpression or gene amplification of HER2, and have expressions greater than 15% of Ki-67 [14, 15]. Compared to the Luminal A subtype, this group responds better to chemotherapy and benefits from hormone therapy [16].

HER2-positive

The HER2-positive tumors are characterized by high expression of HER2 with or without lack of ER and PR. There are two subgroups of this subtype: HER2-positive (HER2-positive, ER-negative, PR-negative, Ki-67>30%) and luminal HER2 (ER-positive, PR-positive, HER2-positive, and Ki-67:15–30%) [17]. The HER2-positive subtype is usually more aggressive than the luminal subtypes and can be treated with chemotherapy and HER2-targeted treatments such as trastuzumab and pertuzumab [18]. Despite being aggressive high-grade tumors, they respond very well to chemotherapy. Additionally, tyrosine kinase inhibitors such as neratinib and lapatinib can also be used [19].

Triple-negative

TNBC subtype consists of ER-negative, PR-negative, and HER2-negative tumors. TNBC can be divided into additional subgroups including basal-like (BL1 and BL2), mesenchymal, claudin-low, luminal androgen receptor, and immunomodulatory. Within these subgroups, the BL1 and BL2 are the most prevalent [20]. Since the triple-negative subtype is not responsive to hormone therapy or targeted therapies, it is more challenging to treat and generally more aggressive than the other subtypes [21]. However, not all TNBCs respond to chemotherapy, and the main reason for treatment failure in TNBC is drug resistance [22]. Moreover, TNBC tumors have increased cell proliferation, DNA repair gene alterations as well as genomic instability [2]. However, there are some benefits from immune checkpoint inhibitors, and researching the mechanism of chemotherapy resistance is especially crucial in TNBC [23].

Genetic Risk Factors for BC

Several genes are found to be associated with the BC development risk. These genes are divided into three groups: high-penetrance, moderate-penetrance, and low-penetrance mutations. The relative risks for developing BC are ≥ 5, between 1.5 and 5, ≥1.01 and <1.5 for high-penetrance, moderate-penetrance, and low-penetrance mutations, respectively [24].

Germline Genetic Mutations in BC

Most of the BCs are sporadic. Hereditary predisposition is responsible for 5–10% of all BCs [25]. Approximately 50% of all cases of familial BC are hereditary [26]. And hereditary disposition to BC is important for patient treatment and follow-ups. Germline mutations in BC can be classified as high, moderate, and low penetrance mutations. These genes and their effect on metabolism will be discussed in further sections.

High Penetrance Mutations

BRCA1 and BRCA2

BC susceptibility gene 1 *(BRCA1)* and *BRCA2* are the most common tumor suppressor genes mutated in BC, and they play a critical part in DNA repair, cell cycle control, and chromosomal integrity in healthy cells [27]. The coding region of *BRCA1* is located on chromosome 17q12-21. It has been linked to over fifteen distinct transcription-related proteins, either in transcriptional repression or activation, and apoptosis. *BRCA1* helps maintain genomic stability as a tumor suppressor. It interacts with several proteins to generate complexes that are important in DNA repair processes and recognition [28-30]. The *BRCA2* gene is found on chromosome 13q12-13. The gene could play a role in the final differentiation of breast epithelial cells and codes for proteins involved in transcription, cell cycle regulation, and DNA repair [28, 29]. Cells with non-functional BRCA1/BRCA2 proteins experience severe impairment in their capacity to repair DNA double-strand breaks (DSBs) [27].

Accordingly, it is well documented that pathogenic variants in *BRCA1/BRCA2* are correlated with the occurrence of BC in both males and females [31]. About 1 in 400 to 800 women in the total population have a germline pathogenic variant in *BRCA1 or BRCA2* [32, 33]. The risk of BC is increased in *BRCA1* and *BRCA2* carriers with a family history of breast cancer compared to the normal population. Women with a mutation in *BRCA1 or BRCA2* and several affected relatives who were diagnosed at a young age have an 80% to 90% lifetime probability of developing BC [34]. Young women are more likely to develop BRCA-associated BC, and BRCA-positive cancers are generally high-grade and hormone receptor-deficient, compared to sporadic disease [35].

In cases where tumors carry deletions in the *BRCA1/BRCA2* genes, they exhibit higher vulnerability against DNA-damaging substances and Poly (ADP-ribose) polymerase family member (PARP) inhibitors [36]. However, a study has examined the clinical outcome of BC patients with *BRCA1* and *BRCA2* variants according to molecular subtypes. Results suggest that the prognostic utility of *BRCA1/BRCA2* germline mutations in BC patients is determined by the molecular subtypes and additionally, with a survival advantage only shown in women with TNBC [37]. This underscores the importance of comprehending the complex network between genetic background and diverse subtypes of BC for better clinical outcomes.

Tumor Protein 53 Gene (TP53)

The tumor protein 53 gene (*TP53*) serves as a tumor suppressor and is responsible for encoding the p53 protein. This gene is located on chromosome 17p13.1. Found within the cell nuclei, The p53 protein directly binds to DNA and reacts to diverse cellular stressors such as chemicals, radiation, and ultraviolet rays from the sun, thereby managing the expression of target genes. Moreover, p53 plays a role in regulating processes like the cell cycle, apoptosis, senescence, and DNA repair [38, 39]. Approximately 30% of BC tumors exhibit mutations in *TP53*, and from a clinical standpoint, the *TP53* status serves as a significant predictive indicator of the response to chemotherapy. [40]. Considering the different subtypes of BC, interestingly, *TP53* is more frequently mutated in ER-negative subtypes than in ER-positive subtypes, ~50% vs~15% respectively.

The Phosphatase Tensin Homolog Gene (PTEN)

The phosphatase tensin homolog gene (*PTEN*) gene encodes tumor suppressor PTEN protein that controls chromosomal integrity, modulates the activity of inositol 1,4,5-trisphosphate receptors, controls apoptosis, transcription, and cell proliferation [41, 42].

PTEN downregulation in BC is linked to an aggressive tumor type, poor prognosis, and lymph node metastases since it triggers the pro-survival pathway PI3K/AKT, which has been shown to be a significant proliferative pathway [43]. PTEN plays a role in the onset and advancement of BC through various mechanisms, such as germline and somatic

mutations in the *PTEN,* loss of heterozygosity at the *PTEN* locus, silencing by methylation of the *PTEN* promoter, protein interactions which decrease *PTEN* transcription, degradation PTEN protein, and post-translational modifications in PTEN protein [44].

Cadherin 1 (CDH1)

Cadherin 1 (*CDH1*) is a tumor suppressor gene that encodes E-cadherin, and functions as a calcium-dependent adhesion molecule facilitating cell-to-cell interactions in epithelial cells [45].

Mutation in the *CDH1* gene can cause invasive lobular carcinoma of the breast [46]. Female mutation carriers of *CDH1* have a 37% lifetime likelihood of developing BC [47].

RAD51C and RAD51D

The genes *RAD51C (FANCO)* and *RAD51D* encode the proteins RAD51C and RAD51D, both of which are part of the RAD51 protein family taking a role in DNA double-strand repair, are essential for processes like non-homologous end joining (NHEJ) and HR. Individuals with germline mutations in *RAD51C* and *RAD51D* have heightened susceptibility to BC, particularly ER-negative BC [4, 48, 49].

STK11

Human Serine/threonine kinase gene (*STK11*) mutations also increase the BC risk [50].

DNA Mismatch Repair (MMR) pathway genes

The last but not the least, DNA Mismatch Repair (MMR) pathway genes including MutL homolog 1 (*MLH1*), mutS homolog 2 (*MSH2*), (*MSH6*), and postmeiotic segregation increased 2 (*PMS2*) cause genome instability and frequently are seen in the patients with hereditary BC [51].

Moderate Penetrate Mutations

Partner and localizer of BRCA2 (PALB2)

Partner and localizer of BRCA2 *(PALB2)* is encoded on chromosome 16p12.2. *PALB2* interacts with *BRCA2* and participates in HR pathways and *PALB2* also increases *RAD51* strand invasion activity [52-54].

Biallelic mutations in *PALB2* cause Fanconi anemia, while monoallelic *PALB2* mutation carriers have an increased risk of developing multiple cancers, especially BC [55-58].

Ataxia-telangiectasia mutated (ATM)

Ataxia-telangiectasia mutated (*ATM*) gene is located on chromosome 11q23 [59]. C 2 is an effector kinase that mediates the response to double-strand DNA breaks in the ATM/CHK2/p53 pathway [60].

ATM gene mutations can cause Ataxia Telangiectasia (AT), which is an autosomal recessive syndrome. Those AT patients who have several symptoms, such as cerebellar neurodegeneration immunodeficiency, telangiectasias, and ionizing radiation sensitivity have also significantly increased risk of cancer, especially BC. *ATM* variants, including V2424G, have the highest risk of BC incidence while *ATM D1853V*, *L546V*, and *S707P* variants have the least effect on BC incidence [59, 61, 62].

The Checkpoint Kinase 2 (CHEK2)

The Checkpoint Kinase 2 (*CHEK2*) gene encodes a checkpoint kinase that is known to interact with DNA repair proteins and regulate the cell cycle [63]. Mutations in the *CHEK2* gene increase susceptibility to BC [4].

BRCA1-associated RING domain (BARD1)

BRCA1 and *BRCA1*-associated RING domain (*BARD1*) interaction is involved in RNA processing, DNA repair (RAD51-mediated HR), regulation of cell cycle, and apoptosis [64].

Low Penetrant Mutations

Neurofibromin 1 (*NF1*) is a tumor suppressor gene and pathogenic variants of the *NF1* gene can cause Neurofibromatosis Type 1 and BC risk is increased in neurofibromatosis type 1 [65-67]. ADP ribosylation factor like GTPase 11 (*ARL11*) is also a tumor suppressor gene that plays a role in several cell regulatory functions, such as cell survival, proliferation, and apoptosis [68]. Mutation in this gene is associated with BC [69]. Some studies associate *ARL11* mutations with poor BC prognosis [70]. Overexpression of mitogen-activated protein kinase 1 E3 ubiquitin-protein ligase (*MAP3K1*) is associated with BC, particularly the luminal subtype with poor prognosis [71]. *MDM2* overexpression is associated with poor prognosis in BC [72]. MDM2 proto-oncogene negatively regulates *TP53*, and overexpression of *MDM2* is associated with

increased risk of developing BC [73, 74]. Moreover, Estrogen Receptor 1 (ESR1) has been linked to a moderately increased risk of developing BC [75]. This mutation is found in hormone receptor-positive BCs and is associated with aromatase inhibitors [76]. Mutations in *ABRAXAS1* are associated with DNA repair defects along with *BRCA1* dysfunction, so there is an elevation in BC risk by impairing *BRCA1* function [77]. Germline mutations in the *PARP4* gene are associated with thyroid cancer as well as BC [78]. Additionally, studies also show that vascular endothelial growth factor (*VEGF*), fibroblast growth factor receptor 2 (*FGFR2*), caspase 8, Lymphocyte-specific protein 1, *TNRC9, H19, TOX* high mobility group box family member 3, are other low penetrant variants associated with BC [79].

The development of BC follows a multi-step process, with each stage linked to specific mutations in critical regulatory genes. The progression model differs between sporadic and hereditary BC. Figure 1 provides an overview of the key genes involved and their roles in both hereditary and sporadic BCs [80].

In conclusion, BC encompasses a wide array of pathological variants, adding to the complexity of the disease. These variants present promising targets for novel drugs, and also exploring new pathological variations can enhance screening initiatives. Moreover, such investigations are vital for unraveling drug resistance mechanisms in BC.

Significant Pathways in BC

BC development is influenced by complex genetic and molecular pathways. Besides, interactions among signaling pathways in cell metabolism have a crucial role in BC development as well. Therefore, concentrating therapeutic efforts on these altered biochemical pathways is essential, as it offers the potential to improve clinical outcomes and advance the creation of effective treatment strategies.

Gene	Locus	Role in Hereditary BC	Role in Sporadic BC
BRCA1	17q12-21	Germline mutation (HBOC syndrome)	Inactivation by hypermethylation of the BRCA1 promotor region
BRCA2	13q12-13	Germline mutation (HBOC syndrome)	Silenced via overexpressed EMSY
PTEN	10q.23-24	Germline mutation (Cowden Disease syndrome)	Rare
TP53	17p13.1	Germline mutation (Li-Fraumeni syndrome) TP53 mutations frequent in BRCA1 and BRCA2 mutant breast cancers	Rare
Rb1	13q14.1	No specific role	Late Event
CDH1	16q22.1	No specific role	Early event in lobular breast
CCND1	11q13	Frequently underexpressed in BRCA1 mutant breast cancers	Overexpressed in 30-40%
MYC	8q24	No specific role	Overexpressed in 25-30%
ERBB2/Her2/neu	17q21	Frequently underexpressed in BRCA1 mutant breast cancers	Overexpressed in 25-30%
ERα	6q25.1	Frequently underexpressed in BRCA1 mutant breast cancers	Underexpressed in 25%
ERβ	14q22-24	Not known	Not known

Figure 1. The key genes and their roles in both hereditary and sporadic BCs

Homologous Recombination Pathway in BC

The cellular genome is continuously exposed to a variety of mutagenic substances, such as oxidants, alkylating agents, ultraviolet light, and ionizing radiation. The maintenance of genomic integrity and DNA repair are ultimately made possible by DNA damage response pathways. Human DNA repair pathways include inter-strand crosslink repair, base excision repair, nucleotide excision repair, MMR, direct damage reversal, and the DSBs repair pathways as HR and NHEJ [81].

Some nucleases, such as the MRE11/RAD50/NBS1 complex, facilitate the formation of 3'-terminal single-stranded DNA (ssDNA) by cleaving the 5'ends of DSBs. The formation of the Rad51 nucleoprotein filament results from the initial binding to the ssDNA by replication protein A, which is later replaced by RAD51. BRCA2 and RAD52 are two important mediators in this process. The RAD51 filaments, with the assistance of PALB2 and RAD51AP1, interact with homologous double-stranded DNA to create a D-loop structure. The D-loop is then disassembled by FANCM, which ends in a non-crossover product. Furthermore, the double Holliday junction structure created during the DSB repair can be resolved by the Bloom syndrome protein (BLM)-Topoisomerase IIIα (TopoIIIα) helicase-topoisomerase complex, producing a non-crossover product [82].

HR deficiency (HRD) is caused by defects in DNA damage repair mechanisms, especially in the HR repair process, which is essential for the repair of DSB [83, 84]. Genomic instability brought on by HRD is a major contributing factor in the development of cancer [83]. Many different mechanisms, such as epigenetic modifications, mutations in genes associated with HR, and indirect interactions between BRCA proteins and other DNA repair proteins, can also cause HRD [85]. HRD is found in various subtypes of primary BC, though its prevalence varies among BC subtypes. In TNBC, HRD is estimated to occur in 50-60% of cases [86]. HRD is less common, but still significant, in hormone receptor-positive (ER-positive/HER2-negative) BCs, ranging from 14–20% [86]. Notably, defects in BRCA1/2 and elevated HRD scores are observed across all BC subtypes, including ER-positive/HER2-negative, ER-negative/HER2-positive, and ER-positive/HER2-positive BC [87]. This finding suggests that HR defects in genes other than *BRCA1/2* are present across all BC subtypes. However, the specific genetic causes of HRD vary by cancer type [83]. For example, almost all triple-negative tumors have methylation in the *BRCA1* promoter region [87]. In conclusion, all other BC subtypes exhibit HRD to varied degrees, although TNBC exhibits the highest frequency of HRD. This finding has important treatment implications. Independent from their BC subtype, patients with HRD tumors may benefit from treatments which target this deficiency, such as PARP inhibitors or platinum-based chemotherapy.

HRD has significant importance in BC treatment, especially for patients with *BRCA1/2* mutations or other defects in the HR repair pathway [88, 89]. Since HRD is linked to sensitivity to DNA-damaging substances like platinum salts and PARP inhibitors, it is an essential biomarker for treatment choices [88, 90]. Remarkably, patients with BC who do not have germline or somatic mutations in *BRCA1/2* or other known HR-related genes can still have HRD. High HRD scores have been associated with mutations in genes, such as *LRP1B, NOTCH3, GATA2,* and *CARD11*, which may increase the number of patients who could benefit from PARP inhibitors and platinum-based chemotherapy [91]. Additionally, the overexpression of certain DNA helicase genes correlates with high HRD scores in both *BRCA*-mutated and *BRCA* wild-type BCs [92].

The phosphatidylinositol 3-kinase (PI3K)/ protein kinase B (AKT)/mammalian target of rapamycin (mTOR) Pathway

One important signaling pathway connected to BC is the PI3K/AKT/mTOR pathway, which is often deregulated in luminal BC [93]. This pathway participates in cell proliferation, migration, apoptotic cell death, DNA repair mechanism and glucose metabolism. It increases angiogenesis, survival, growth, and proliferation of BC cells [94]. Even in normoxic settings, the altered PI3K/AKT/mTOR pathway in BC cells are able to increase hypoxia-inducible factor 1 alpha (HIF-1α), which activates genes related to glycolysis and glucose uptake. Further, tumor development and carcinogenesis are influenced by HIF-1α [95]. Additionally, Hexokinase II, which is essential for cancer cells to survive and adjust to the varying cellular environment, is upregulated by PI3K/AKT/mTOR pathway [96]. AKT phosphorylates numerous cellular proteins, such as MDM2, FOXO, BCL2-interacting mediator of cell death, BCL2-associated

agonist of cell death, and glycogen synthase kinases 3α (GSK3α) and 3β (GSK3β), in order foster cell survival and promote cell cycle progression [97]. Additionally, the PI3K/AKT/mTOR pathway is a major contributor to resistance against standard therapies in BC. It has emerged as a novel target for overcoming drug resistance in recent years [98].

Wnt Signaling Pathway

The Wnt signaling pathway controls the capacity of self-renewal and differentiation potential of stem cells in humans [99]. There are two types of Wnt signaling pathways: canonical and noncanonical. β-catenin is the main effector molecule in the canonical Wnt signaling pathway. β-catenin interacts with transcription factors belonging to the T cell factor/lymphoid-enhanced binding factor 1 (TCF/LEF1) family. The key regulator steps in this pathway are the stabilization and presence of β-catenin in the cytoplasm, and then its translocation into the cell nucleus, so it can bind to TCF/LEF1 and triggers target gene expression [100]. Without the Wnt signal, the axin-APC complex anchors β-catenin, which is subsequently phosphorylated by casein kinase Iα (CKIα) and GSK3β. The 26S proteasome degrades β-catenin as a result of this phosphorylation, which prepares it for ubiquitination by the SKP1–cullin1–F-box (SCF-β-TRCP) E3 ubiquitin ligase. Wnt inactivates the axin-APC destruction complex by recruiting the intracellular signaling protein dishevelled (DVL) after binding to the frizzled (FZD) and LRP5/6 coreceptors. This mechanism prevents β-catenin from degrading, enabling it to enter the nucleus and initiate transcription [101, 102]. Noncanonical Wnt signaling pathways are independent of β-catenin. The PCP pathway and the Ca^{2+} pathway are the two primary types of these pathways. In order to initiate non-canonical Wnt signaling, Wnt5a and Wnt11 bind to different receptors such as RYK, ROR2, ROR1, or the Fzd family [103, 104].

Dysregulation of the Wnt signaling pathway is common in various cancer types, and it is involved in BC development and progression like other cancers [105]. Each of the Wnt/β-Catenin, Wnt-PCP, and Wnt-Ca^{2+} signaling pathways contribute differently to the development of BC and share overlapping components [106].

Genome-wide sequencing and gene expression profile analysis has demonstrated that the Wnt signaling pathway has an important part in BC metastasis and proliferation [106]. Wnt signaling is also crucial for maintaining stemness, regulating the immune microenvironment of BC, producing treatment resistance, and developing cancer phenotypes, according to recent research [106]. Furthermore, the WNT/β-Catenin pathway has a role in the development of TNBC [107, 108].

WNT signaling causes the shifting to Warburg phenotype in BC cells by upregulating phosphofructokinase platelets (PFKP). Furthermore, WNT3A triggers the activation of the epidermal growth factor receptor (EGFR), which in turn activates the PI3K/Akt pathway. This activation enhances the glycolytic phenotype, cell division, and migration potential of cancer cells by phosphorylating and stabilizing PFKP [109, 110]. Studies show that the WNT β-catenin pathway increases the M2 isoform of pyruvate kinase in BC cells, which increases glycolysis and stemness in BC cells [111]. Additionally, the canonical WNT/β-catenin signaling pathway suppresses mitochondrial respiration by reducing the ATP synthase subunit expression, cytochrome c oxidase, and cytochrome c1. As a result, aerobic glycolysis increases [112, 113].

The Wnt signaling pathway interacts with a variety of proteins. The adenomatous polyposis coli (APC) gene serves as a vital regulator within the Wnt pathway, functioning as a tumor suppressor gene [106]. A transcription factor called ΔNp63 increases the expression of the Wnt receptor Frizzled Class Receptor 7 (Fzd7), which in turn increases the activities of mammary stem cells. BC stem cells (BCSCs) use this certain mechanism as well, to facilitate tumor growth in TNBC [114].

The Wnt signaling pathway can also be controlled by micro-RNAs (miRNAs). The levels of Lethal-7 (let-7) miRNAs are dramatically reduced in the MCF-7 BC cell line and reintroducing them can suppress the proliferation of BC cells [115]. Stearoyl-Coenzyme A desaturase 1, an enzyme necessary for Wnt signaling activation, is the direct target of miR-600. Inhibiting miR-600 results in the proliferation of BCSCs, while increasing its expression might reduce BCSC self-renewal [116, 117]. In addition, BCSCs go into a quiescent state when Wnt signaling becomes inactivated, which makes them drug-insensitive and leads to multidrug resistance in BC [118].

Moreover, AF1q, a protein associated with poor prognosis in various cancers, especially BC, has been linked to the activation of the Wnt signaling pathway. Protein AF1q increases BC cells' ability to form spheres by promoting the development of stem-like populations via the activation of the Wnt pathway [119]. This finding suggests the relationship between cancer stem cells, Wnt signaling, and the development of BC.

In summary, the Wnt signaling pathway exerts a significant role in BC development and affects many aspects of tumor biology. It may be achievable to improve treatment options by comprehending the mechanisms of Wnt signaling in BC [106]. One possible treatment strategy for BC is to target particular Wnt pathway components, such as CDK14, which is upregulated in TNBC [120].

Nuclear factor κ-B (NF-κB) Pathway

The non-canonical NF-κB pathway increases the expression of Indoleamine 2,3-dioxygenase (IDO). Higher IDO activity contributes to immunosuppression, tumor metastasis, and is associated with poor prognosis [121, 122].

Lactic acid, formed as a result of anaerobic respiration in cancer cells, is released outside the cell via MCT4 and taken into endothelial cells via MCT1, which causes tumor angiogenesis via the NF-κB/IL-8 pathway [123]. In BCs with BCL2 overexpression, the anti-apoptotic effect of BCL2 is enhanced by NF-κB [124].

Notch Signaling Pathway

The Notch pathway is a highly conserved cell signaling pathway in eukaryotes. The Notch signaling pathway is activated in both malignant and normal stem cells, and it is essential for stem cell maintenance, differentiation, proliferation, and cell fate determination. Normal stem cells are able to maintain a balance between self-renewal, differentiation, and proliferation through interactions with various signaling networks, including the JAK-STAT pathway [125]. Likewise, the abnormal stimulation of Notch signaling pathways facilitates the self-renewal, cell proliferation, and metastasis of BCSCs [126]. Therefore, it is considered that the Notch signaling pathway is essential for determining the destiny of BCSCs [127]. The invasion, mesenchymal-like characteristics, and drug resistance of BCSCs can be stimulated and maintained via the Notch pathway via JAG-1 and NOTCH-4 [128, 129]. MAP 17 (PDZKIP1) stimulates the Notch pathway, inhibits NUMB activity, and encourages BCSC maintenance [130]. The NUMB protein suppresses the Notch pathway and blocks the Notch intracellular domain (NICD) in the cytoplasm of non-cancerous cells. miR-146a stimulates the Notch pathway, inhibits NUMB activity, and causes BCSCs to proliferate [131]. Notch signaling induces stemness by promoting the deacetylation and subsequent activation of ALDH1A1 [132]. Furthermore, there is a high correlation between the Notch signal and BCSC Ki-67 expression [133]. The normal epithelial cell transition into malignant BC cells is also induced by the Notch pathway [134]. The pathway's ability to crosstalk with other signaling systems, such as PI3K/AKT, NF-κB,, and miRNAs, further emphasizes its importance in precisely regulating cell fate [135]. In addition, a study suggests that Notch signaling regulates NF-κB activity and mitochondrial metabolism in TNBC cells through IKKα-Dependent Non-Canonical pathways. [136]. Since utilizing inhibitors that target the Notch signaling pathway has demonstrated effectiveness in reducing the BSCS population, BC treatment responsiveness may be enhanced by inhibitors of the Notch signaling [137]. Besides, in TNBC cases with mutated *Notch1* and wild-type *PTEN* expression, combination therapies that target the intersection of the Notch, AKT, and NF-κB pathways may also have therapeutic uses in regard to BCSCs [136].

HIF-1 Alfa Pathway

HIFs-mediated downstream pathways, mainly VEGF, can trigger angiogenesis in BC [138]. It is shown that proline residues P402 and P564 of the human HIF-1α ODD domain are hydroxylated by prolyl hydroxylase domain proteins (PHDs), under normoxic conditions [139]. This hydroxylation process is essential for the HIF-1α protein to bind the von Hippel-Lindau protein (pVHL) [140]. As the recognition subunit of an E3 ubiquitin ligase, VHL attaches a poly-ubiquitin chain to HIF-1α, directing the proteasome to degrade HIF-1α [141]. Transactivation function of HIF-1α under normoxic conditions is also inhibited by Factor İnhibiting HIF-1 (FIH-1)'s oxygen-dependent hydroxylation of the asparagine residue N803 in HIF-1α, which also prevents HIF-1α interactions with the coactivators p300 and CBP (CREB binding protein) [142].

HIF-1α can reduce the expression of the tricarboxylic acid (TCA) enzymes, accelerate glycolysis and lactic acid accumulation, and result in immunosuppression and angiogenesis [143]. HIF-1α increases glucose uptake by cancer cells through increasing the expression of glucose transporter 1 (GLUT1) [144]. Moreover, HIF-1α elevates pyruvate dehydrogenase kinase (PDK) activity, which suppresses pyruvate dehydrogenase (PDH). This inhibition leads to a decreased flux of pyruvate into TCA. In addition, HIF-1α raises lactate dehydrogenase (LDH) activity, which converts pyruvate to lactate [145].

α-Ketoglutarate (αKG) Signaling

Within tumor cells bearing Isocitrate dehydrogenase mutations, there is a reduction in α-KG levels, resulting in heightened levels of HIF-1α and a greater recurrence rate [146]. Elevated α-KG levels are linked to reduced tumor metastasis as a result of augmented DNA demethylation, hindered cell migration, and the downregulation of Zeb1 [147]. Despite indications of the potential tumor-suppressive effects of αKG, its regulatory impact on BC is not yet fully understood, emphasizing the necessity for further research to validate this role.

STAT3 Pathway

Signal transducer and activator of transcription 3 (STAT3) is a tumor diagnostic marker and is known to increase BC malignancy [148]. The activation of STAT3 is triggered by different receptors, including the IL-6 receptor, tyrosine kinase receptors, fibroblast growth factor receptors, and platelet-derived growth factor receptors. In addition, toll-like receptors, G protein-coupled receptors, and receptors like EGFR, SRC, and ABL are involved in this process [149].

It is known that the increased activity of the STAT3 pathway is linked to increased BC progression, proliferation, metastasis, and chemoresistance with decreased apoptosis [148]. Upregulation of STAT3 increases IDO expression through the non-canonical NF-κB pathway, leading to tumor metastasis [150]. In cancer cells, STAT proteins promote aerobic glycolysis via upregulating the expression of MYC and HIFs [151]. These factors are important drivers of the Warburg effect, enhancing the expression of genes that encode main glycolytic enzymes and proteins which are responsible for glucose uptake. Additionally, STAT3 specifically regulates several glycolysis-related genes, including *HK2, PKM, SLC2A1* (which encodes GLUT1), *SLC2A3* (which encodes GLUT3) and enolase 1 (*ENO1)* [152]. Furthermore, in mitochondria, STAT3 is involved in controlling the activity of the electron transport chain, which produces reactive oxygen species (ROS) [149]. As a result, treatment with STAT3 inhibitors alone or combined with other therapeutic medications may have more encouraging results when it comes to reducing or eliminating chemoresistance in BC.

RHOA/ROCK/GLUT1 pathway

TP53, can induce cell cycle arrest, apoptosis, and control tumor cell metabolism by inhibiting the effect of *TP53* on glycolysis through the suppression of GLUT1, GLUT3, and GLUT4 expression and the regulation of the enzymatic expression of HK2, PFK1, PDH, PDK2, phosphoglycerate mutase, and parkin 2 [153]. Mutated *TP53* contributes to tumorigenesis through increasing glycolysis via activating the RhoA/ROCK/GLUT1 signaling pathway, since this pathway causes the GLUT1 translocation to the plasma membrane [154].

SNAIL/E-Cadherin Pathway

The transcription factor SNAIL induces epithelial-to-mesenchymal transition (EMT), and its inhibition promotes mesenchymal-to-epithelial transition breast epithelial cells, enhancing cancer stem cell-like characteristics [155]. Phosphoglucoisomerase (PGI)/Autocrine motility factor (AMF) is linked to the downregulation of epithelial markers, such as E-cadherin, through the SNAIL/E-Cadherin pathway [156]. Moreover, the high expression of Snail and low expression of E-cadherin is found in adriamycin-resistant human BC MCF-7/ADM cells [157]. These point towards a promising research direction for targeted drug-resistant BC therapy, which could potentially provide valuable clinical guidance for BC therapy and prognosis evaluation.

As a summary, we highlighted the key genetic pathways in BC in Table 1.

Metabolic Alterations in BC

In recent years, extensive focus in cancer research has shifted towards understanding the dysregulation of cellular metabolism within cancer cells, as it is now recognized as a key hallmark of cancer. Growing evidence suggests that the disrupted cellular metabolism could significantly

Table 1. Key genetic and molecular pathways in BC

Pathway	Role in Cancer
HER2/ERBB2 Pathway	Overexpression or amplification of the HER2/ERBB2 gene leads to aggressive tumor growth and resistance to apoptosis.
Hormone Receptor (ER/PR) Pathway	Mutations or overexpression can drive hormone-dependent BC proliferation.
PI3K/AKT/mTOR Pathway	Mutations in PIK3CA or loss of tumor suppressor PTEN activate this pathway, promoting growth and therapy resistance.
TP53 Pathway	Mutations in TP53 are common in aggressive BCs, particularly triple-negative subtypes.
BRCA1/BRCA2 Pathway	Mutations increase the risk of breast and ovarian cancers by impairing DNA repair.
Wnt/β-Catenin Pathway	Dysregulation, often through mutations or overexpression, contributes to tumor progression and metastasis.
Notch Pathway	Overactivation promotes tumorigenesis, stemness, and therapy resistance.
NF-κB Pathway	Chronic activation supports cell survival, proliferation, and angiogenesis.
MYC Pathway	Amplification or overexpression leads to uncontrolled proliferation and metabolic reprogramming.

contribute to the development of drug resistance in cancer patients. According to the principles of the Warburg effect, cancer cells exhibit a preference for glycolysis whether there is oxygen availability or not, indicating the presence of mitochondrial dysfunction [7]. This metabolic shift is illustrated in Figure 2. The other theory, "the reverse Warburg effect" indicates that cancer cells enhance aerobic glycolysis in tumor-associated fibroblasts, and lactate and pyruvate produced by these cells promote tumor growth and development [158]. This concept of metabolic reprogramming encompasses not only glucose metabolism but also extends to lipid and amino acid metabolism [6]. The observed metabolic reprogramming in resistant BC cells holds substantial therapeutic promise. This highlights the opportunity to exploit metabolic vulnerabilities for therapeutic advantages in BC management.

Altered Glucose Metabolism in BC

The reprogramming of glucose metabolism in cancers facilitates the energy needs of rapidly growing cancer cells. Abnormal expressions of glycolytic-related enzymes can promote oncogenesis, support tumor growth, and contribute to treatment resistance. Studies have shown that key glycolytic enzymes such as HK, PFK, ENO, PK, and LDH are upregulated in BC, as well as GLUTs [159-162]. However, molecular and metabolic heterogeneity are characteristics of BC. While TNBC is linked to the Warburg effect and mixed types, the luminal-A subtype often displays the reverse Warburg effect [163]. Studies also show that wild-type *TP53* inhibits glycolytic activity and enhances oxidative phosphorylation by reducing the expression of glycolytic enzymes and increasing the levels of mitochondrial proteins. Accordingly, cancer cells lacking functional p53 tend to exhibit

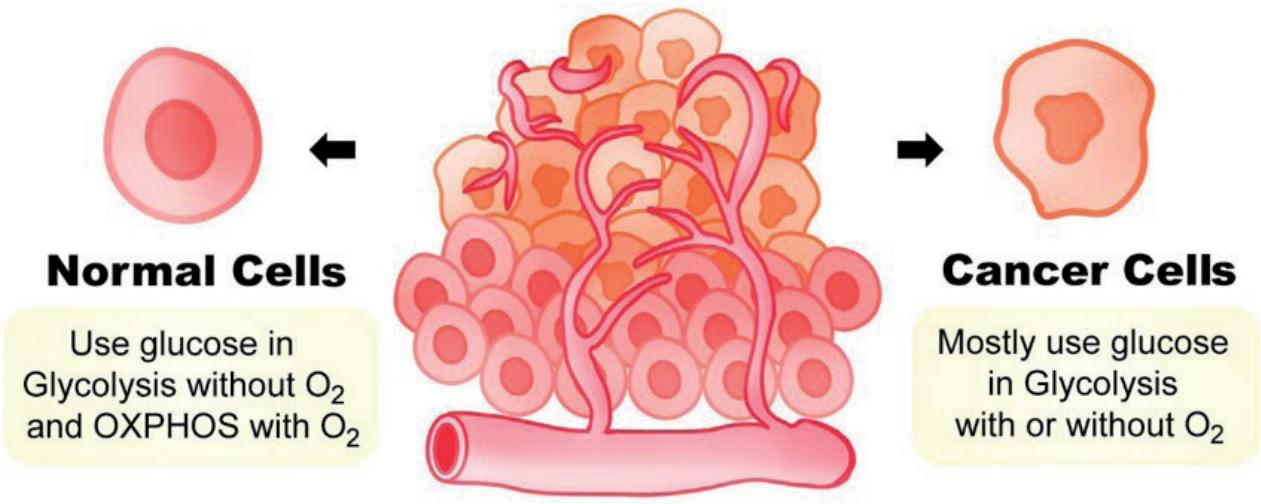

Figure 2. The warburg effect in cancer cell metabolism

metabolic reprogramming towards glycolysis, leading to an increased reliance on this pathway for energy production. This highlights the significance of the diverse molecular features present in various subtypes of BC, which contribute to the metabolic diversity observed within the disease [164].

It is known that GLUT1-mediated glucose uptake is a pivotal component in the development of BC, as the loss of a single copy of SLCA2A1, which encodes GLUT1, is adequate to prevent the neoplastic process of the Neu-induced breast tumor *in vivo* [165]. Moreover, ectopic overexpression of GLUT1 and GLUT3 has been reported to be associated with chemotherapy resistance in BC cells [166]. In line with this, TNBC which is known as the most aggressive subtype of BC, demonstrates elevated expression of GLUT1 compared to non-TNBC [167]. Compared to other subtypes, ER-positive BCs depend less on glucose uptake, favoring the consumption of lactate produced by neighboring cancer-associated fibroblasts (CAFs) [9]. Moreover, HIF-1 can also accelerate glycolysis by regulating glycolytic pathway enzymes, including HK2, LDHA, and GLUT1 as well as by reducing the expression of TCA enzymes [143].

The Pentose Phosphate Pathway (PPP) is an alternative pathway for glucose oxidation in addition to glycolysis. Due to the critical role of the PPP in facilitating tumor proliferation and enabling cancer cells to endure the impacts of ROS, elevated levels of certain PPP enzymes, like glucose 6-phosphate dehydrogenase and transketolase, are also correlated with poor outcomes in BC [168]. Some PPP enzymes, however, are primarily expressed in HER2-positive tumors, indicating that activation of PPP is fundamental in this intrinsic subtype of BC [169].

Glucose not only participates in glycolysis or the PPP but also contributes to the hexosamine biosynthetic pathway (HBP). The HBP ultimately leads to the synthesis of UDP-GlcNAc, an amino sugar that, along with other nucleotide sugars, forms the foundation for glycoprotein and glycoconjugate biosynthesis [170]. Additionally, O-GlcNAc can indirectly regulate transcription by impacting cancer metabolism. In BC cells, increased O-GlcNAcylation leads to a decrease in the TCA metabolite α-KG, resulting in reduced hydroxylation of HIF-1α and its interaction with the pVHL. As a consequence, HIF-1α is stabilized, leading to enhanced expression of its transcriptional targets, such as GLUT1, and it contributes significantly to the survival of BC cells under metabolic stress [171]. Overall metabolic alterations in different BC subtypes are summarized in Figure 3 [9]. Consequently, the comprehensive metabolic reprogramming in different BC subtypes remains to be fully revealed.

Altered Lipid Metabolism in BC Tumors

The *de novo* synthesis of fatty acids is a crucial metabolic characteristic that sets cancer cells apart from normal cells. Even though it is seen in normal cells, it is restricted to the liver, adipose tissue, and breast during lactation. Meeting the increased need for membrane production, the metabolism of fatty acids (FAs) and lipids plays a significant role in promoting the growth and progression of BC [172]. Research has demonstrated that several key enzymes involved in lipid metabolism in breast tumors, including acetyl-CoA carboxylase (ACC),

	Breast Cancer Subtypes		
Metabolic alterations	**ER+ (Luminal A, Luminal B)**	**HER+**	**TNBC**
Glycolytic Flux	Context dependent	Increased	Increased
Glutamine Catabolism	Decreased	Increased	Increased
Lipid Metabolism	Increased cholesterol biosynthesis	Increased Fatty acid biosynthesis	Increased cholesterol uptake

Figure 3. Overall metabolic alterations in BC subtypes

ATP citrate lyase, monoacylglycerol lipase (MAGL), and fatty acid synthase (FASN), are upregulated [153]. Inhibition of these enzymes can hinder tumor growth and metastasis. Notably, ACC, the enzyme that governs the rate of fatty acid synthesis, is highly expressed in BC, and inhibition of ACC results in increased cell apoptosis [173]. Furthermore, a study has reported an interaction between BRCA1 and ACC-alpha (ACCα) through the BRCA1 C-Terminal domain. Variations in the *BRCA1* gene may cause a disruption of the BRCA1-ACCα complex, which leads to increase in ACCα release and lipogenesis in breast tumor cells [174].

Additionally, the dysregulation of Acyl-coenzyme A synthetase short-chain family member 2 (ACSS2) in cancer, particularly BC, has been associated with a poorer prognosis. ACSS2 is often highly expressed in BC and acts as a metabolic immunomodulator, thereby influencing cancer progression [175].

Another enzyme found to have a role in BC pathogenesis is MAGL. MAGL is an enzyme that has shown involvement in tumor progression through energy supply by fatty acid oxidation and increased oncogenic signaling lipids like free fatty acids, monoacylglycerol, and secondary lipid metabolites. These promote migration, invasion, survival, and in vivo tumor growth, leading to increased malignancy of cancer cells [176].

The FASN enzyme can be directly activated by HER2, leading to the expression of FASN in BC cells [177]. In contrast, TNBCs demonstrate lower levels of FASN expression. As a result, HER2-positive BCs increase the *de novo* production of lipids, while TNBCs increase their lipid uptake [178]. The differences in lipid metabolism in BC subtypes are summarized in Figure 3 [9].

In BC, disruptions in lipid metabolism can result in the accumulation of free fatty acids and cholesterol within the tumor microenvironment. This accumulation has been observed to negatively impact the activation and function of immune cells. Elevated levels of free fatty acids have been linked to impaired immune function in CD8+ T cells, while increased cholesterol levels have been shown to inhibit T cell receptor signaling, causing impaired T cell proliferation and cytokine production. These effects eventually contribute to a weakened anti-tumor immune response. Moreover, increased free fatty acid levels may stimulate the production of myeloid-derived suppressor cells and immunosuppressive regulatory T cells, which efficiently inhibit the activity of effector immune cells and impede immune responses against cancer [179].

Therefore, investigating the regulatory mechanism of fatty acid synthesis and its effect on various tumor subtypes can be helpful for an accurate understanding of tumor pathogenesis and the development of more effective strategies for treatment.

Amino Acid Metabolism Alterations in BC

In BC, glutamine metabolism is significantly altered, which has a significant impact on the metabolism of amino acids. A nonessential amino acid, glutamine is necessary for many metabolic processes, including nucleotide biosynthesis and protein synthesis [180]. Glutamate dehydrogenase, cell-surface glutamine transporter ASCT2, and glutaminase-1 are among the proteins linked to glutamine metabolism that have been found to express more in HER2-positive BC than in other subtypes. This implies that there is increased glutamine metabolism activity in HER2-positive BC [181]. Compared to HER2-positive and luminal subtypes, TNBC tumors exhibit significantly higher expression of the glutaminase enzyme, which transforms glutamine into glutamic acid [181]. Therefore, exogenous glutamine is necessary for the TNBC cells to survive [182]. Not because they proliferate less, but rather because the luminal tumors themselves can synthesize glutamine through the expression of a glutamine-synthetase enzyme, these subtypes are less reliant on exogenous glutamine [54]. The differences between glutamine metabolism across different BC subtypes are shown in Figure 3.

Serine plays a vital role in providing one-carbon units crucial for DNA synthesis for cellular proliferation. Alongside the upregulation of glutamine metabolism, the increased activity in serine metabolism is associated with the heightened proliferation of tumor cells and is indicative of a poor prognosis for patients. 3-phospho-glycerate-dehydrogenase, the initial enzyme involved in serine synthesis, is fundamentally overexpressed in BC, particularly in subtypes characterized by higher proliferation rates, such as ER-negative

tumors [183, 184]. Nevertheless, these amino acids do not only participate in biosynthesis but also communicate with signaling pathways. For instance, glutamine activates mTORC1 signaling and leads to tumor proliferation [185]. Overall, these findings underscore that understanding the interplay between metabolic pathways and the distinct metabolic reprogramming across different subtypes is essential for more efficient BC therapies.

BC Heterogeneity and Immunotherapy

Tumors are recognized to adopt diverse strategies to avoid immune detection and clearance by the immune system, such as activating inhibitory pathways controlled by immune checkpoints. The administration of immune checkpoint inhibitors (ICIs) disrupts these inhibitory signals, revitalizing the anti-tumor immune response, as validated by a multitude of studies and clinical trials utilizing monoclonal antibodies targeting programmed death-1 (PD-1), programmed death ligand-1 (PD-L1), and cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) [186].

The heterogeneity of breast tumors prompts the question of whether specific types of breast tumors may derive greater benefit from immune-based treatments, and what cellular or environmental factors within the cancer cells contribute to the likelihood of eliciting a strong and lasting anti-tumor immune response [187]. It has been suggested that BC is an immune-silent type of cancer that is less responsive to immunotherapy. Yet, mounting evidence suggests that BC encompasses a diverse range of tumors with varying levels of immunogenicity. In this spectrum, TNBC is thought to represent a particularly immunogenic subtype, and treatment with ICI has been shown to enhance clinical outcomes [188, 189]. Currently, a significant portion of BC research is dedicated to inhibiting the PD1/PD-L1 axis. A study delving into the concurrent use of PD1/PD-L1 and CTLA-4 inhibitors demonstrated a noteworthy tumor size reduction in metastatic TNBC patients with a %43 objective response rate. Intriguingly, individuals with HR-positive BC did not exhibit any responses to this combination treatment [190]. Moving forward, combining ICIs with chemotherapy, PARP inhibitors, or other therapies shows promising potential for enhancing the clinical efficacy in TNBC. However, to maximize the benefits of these treatments, it will be essential to identify reliable predictive biomarkers for patient selection. This emphasis on predictive biomarkers and understanding the tumor microenvironment paves the way for more precise and effective interventions in the future.

Conclusions

In conclusion, a comprehensive understanding of BC's genetic and metabolic features is essential for developing more effective treatment strategies. As research continues to elucidate the complex interactions among these molecular factors, the development of personalized and targeted therapies will be crucial in addressing the challenges posed by this heterogeneous disease. Integrating insights from genetic studies, signaling pathways, and metabolic reprogramming will pave the way for more precise and effective interventions, ultimately improving survival rates and quality of life for BC patients. The highlighted molecular pathways in this review can help us discover novel diagnostic and prognostic biomarkers and hopefully, new therapy targets to overcome drug resistance and off-target side effects. By using these biomarkers, eventually, we will also maximize the efficacy of current treatments and minimize their toxicities.

Author contribution

Study conception and design: YD, AB, and BC; draft manuscript preparation: YD, AB, and BC. All authors reviewed the results and approved the final version of the manuscript.

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REFERENCES

[1] Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229-63.

[2] Orrantia-Borunda E, Anchondo-Nunez P, Acuna-Aguilar LE, Gomez-Valles FO, Ramirez-Valdespino CA. Subtypes of Breast Cancer. In: Mayrovitz HN, editor. Breast Cancer. Brisbane (AU)2022.

[3] Claus EB, Schildkraut JM, Thompson WD, Risch NJ. The genetic attributable risk of breast and ovarian cancer. Cancer. 1996;77(11):2318-24.

[4] Breast Cancer Association C, Dorling L, Carvalho S, Allen J, Gonzalez-Neira A, Luccarini C, et al. Breast Cancer Risk Genes - Association Analysis in More than 113,000 Women. N Engl J Med. 2021;384(5):428-39.

[5] Mavaddat N, Michailidou K, Dennis J, Lush M, Fachal L, Lee A, et al. Polygenic Risk Scores for Prediction of Breast Cancer and Breast Cancer Subtypes. Am J Hum Genet. 2019;104(1):21-34.

[6] Zheng X, Ma H, Wang J, Huang M, Fu D, Qin L, et al. Energy metabolism pathways in breast cancer progression: The reprogramming, crosstalk, and potential therapeutic targets. Transl Oncol. 2022;26:101534.

[7] Bose S, Zhang C, Le A. Glucose Metabolism in Cancer: The Warburg Effect and Beyond. Adv Exp Med Biol. 2021;1311:3-15.

[8] Lv L, Yang S, Zhu Y, Zhai X, Li S, Tao X, et al. Relationship between metabolic reprogramming and drug resistance in breast cancer. Front Oncol. 2022;12:942064.

[9] Ogrodzinski MP, Bernard JJ, Lunt SY. Deciphering metabolic rewiring in breast cancer subtypes. Transl Res. 2017;189:105-22.

[10] Johnson KS, Conant EF, Soo MS. Molecular Subtypes of Breast Cancer: A Review for Breast Radiologists. J Breast Imaging. 2021;3(1):12-24.

[11] Couture HD, Williams LA, Geradts J, Nyante SJ, Butler EN, Marron JS, et al. Image analysis with deep learning to predict breast cancer grade, ER status, histologic subtype, and intrinsic subtype. NPJ Breast Cancer. 2018;4:30.

[12] Vuong D, Simpson PT, Green B, Cummings MC, Lakhani SR. Molecular classification of breast cancer. Virchows Arch. 2014;465(1):1-14.

[13] Higgins MJ, Stearns V. Understanding resistance to tamoxifen in hormone receptor-positive breast cancer. Clin Chem. 2009;55(8):1453-5.

[14] Inic Z, Zegarac M, Inic M, Markovic I, Kozomara Z, Djurisic I, et al. Difference between Luminal A and Luminal B Subtypes According to Ki-67, Tumor Size, and Progesterone Receptor Negativity Providing Prognostic Information. Clin Med Insights Oncol. 2014;8:107-11.

[15] Al-Thoubaity FK. Molecular classification of breast cancer: A retrospective cohort study. Ann Med Surg (Lond). 2020;49:44-8.

[16] Lafci O, Celepli P, Seher Oztekin P, Kosar PN. DCE-MRI Radiomics Analysis in Differentiating Luminal A and Luminal B Breast Cancer Molecular Subtypes. Acad Radiol. 2023;30(1):22-9.

[17] Krishnamurti U, Silverman JF. HER2 in Breast Cancer: A Review and Update. Advances in Anatomic Pathology. 2014;21(2):100-7.

[18] Oh DY, Bang YJ. HER2-targeted therapies - a role beyond breast cancer. Nat Rev Clin Oncol. 2020;17(1):33-48.

[19] Figueroa-Magalhaes MC, Jelovac D, Connolly R, Wolff AC. Treatment of HER2-positive breast cancer. Breast. 2014;23(2):128-36.

[20] Kumar P, Aggarwal R. An overview of triple-negative breast cancer. Arch Gynecol Obstet. 2016;293(2):247-69.

[21] Garrido-Castro AC, Lin NU, Polyak K. Insights into Molecular Classifications of Triple-Negative Breast Cancer: Improving Patient Selection for Treatment. Cancer Discov. 2019;9(2):176-98.

[22] Nedeljkovic M, Damjanovic A. Mechanisms of Chemotherapy Resistance in Triple-Negative Breast Cancer-How We Can Rise to the Challenge. Cells. 2019;8(9).

[23] Singh DD, Yadav DK. TNBC: Potential Targeting of Multiple Receptors for a Therapeutic Breakthrough, Nanomedicine, and Immunotherapy. Biomedicines. 2021;9(8).

[24] Foulkes WD. Inherited susceptibility to common cancers. N Engl J Med. 2008;359(20):2143-53.

[25] Kleibl Z, Kristensen VN. Women at high risk of breast cancer: Molecular characteristics, clinical presentation and management. Breast. 2016;28:136-44.

[26] Wooster R, Weber BL. Breast and ovarian cancer. N Engl J Med. 2003;348(23):2339-47.

[27] Roy R, Chun J, Powell SN. BRCA1 and BRCA2: different roles in a common pathway of genome protection. Nat Rev Cancer. 2011;12(1):68-78.

[28] Cable PL, Wilson CA, Calzone FJ, Rauscher FJ, 3rd, Scully R, Livingston DM, et al. Novel consensus DNA-binding sequence for BRCA1 protein complexes. Mol Carcinog. 2003;38(2):85-96.

[29] Jhanwar-Uniyal M. BRCA1 in cancer, cell cycle and genomic stability. Front Biosci. 2003;8:s1107-17.

[30] Venkitaraman AR. Cancer susceptibility and the functions of BRCA1 and BRCA2. Cell. 2002;108(2):171-82.

[31] Malone KE, Daling JR, Doody DR, Hsu L, Bernstein L, Coates RJ, et al. Prevalence and predictors of BRCA1 and BRCA2 mutations in a population-based study of breast cancer in white and black American women ages 35 to 64 years. Cancer Res. 2006;66(16):8297-308.

[32] Anglian Breast Cancer Study G. Prevalence and penetrance of BRCA1 and BRCA2 mutations in a population-based series of breast cancer cases. Anglian Breast Cancer Study Group. Br J Cancer. 2000;83(10):1301-8.

[33] Maxwell KN, Nathanson KL. Common breast cancer risk variants in the post-COGS era: a comprehensive review. Breast Cancer Res. 2013;15(6):212.

[34] Kurian AW, Kingham KE, Ford JM. Next-generation sequencing for hereditary breast and gynecologic cancer risk assessment. Current Opinion in Obstetrics and Gynecology. 2015;27(1):23-33.

[35] Bordeleau L, Panchal S, Goodwin P. Prognosis of BRCA-associated breast cancer: a summary of evidence. Breast Cancer Res Treat. 2010;119(1):13-24.

[36] Davey MG, Davey CM, Ryan EJ, Lowery AJ, Kerin MJ. Combined breast conservation therapy versus mastectomy for BRCA mutation carriers - A systematic review and meta-analysis. Breast. 2021;56:26-34.

[37] De Talhouet S, Peron J, Vuilleumier A, Friedlaender A, Viassolo V, Ayme A, et al. Clinical outcome of breast cancer in carriers of BRCA1 and BRCA2 mutations according to molecular subtypes. Sci Rep. 2020;10(1):7073.

[38] Patocs A, Zhang L, Xu Y, Weber F, Caldes T, Mutter GL, et al. Breast-cancer stromal cells with TP53 mutations and nodal metastases. N Engl J Med. 2007;357(25):2543-51.

[39] Zhang W, Edwards A, Flemington EK, Zhang K. Significant Prognostic Features and Patterns of Somatic TP53 Mutations in Human Cancers. Cancer Inform. 2017;16:1176935117691267.

[40] Li X, Niu Z, Sun C, Zhuo S, Yang H, Yang X, et al. Regulation of P53 signaling in breast cancer by the E3 ubiquitin ligase RNF187. Cell Death Dis. 2022;13(2):149.

[41] Pulido R, Baker SJ, Barata JT, Carracedo A, Cid VJ, Chin-Sang ID, et al. A unified nomenclature and amino acid numbering for human PTEN. Sci Signal. 2014;7(332):pe15.

[42] Lee YR, Chen M, Pandolfi PP. The functions and regulation of the PTEN tumour suppressor: new modes and prospects. Nat Rev Mol Cell Biol. 2018;19(9):547-62.

[43] Kamal A, Awan AR, Rabbani M, Sheikh HR, Tayyab M, Firyal S, et al. The interplay of PTEN and AKT nexus in breast cancer: a molecular perspective. Mol Biol Rep. 2024;51(1):345.

[44] Kechagioglou P, Papi RM, Provatopoulou X, Kalogera E, Papadimitriou E, Grigoropoulos P, et al. Tumor Suppressor PTEN in Breast Cancer: Heterozygosity, Mutations and Protein Expression. Anticancer Research. 2014;34(3):1387.

[45] Christofori G, Semb H. The role of the cell-adhesion molecule E-cadherin as a tumour-suppressor gene. Trends Biochem Sci. 1999;24(2):73-6.

[46] Cowin P, Rowlands TM, Hatsell SJ. Cadherins and catenins in breast cancer. Curr Opin Cell Biol. 2005;17(5):499-508.

[47] Ryan CE, Fasaye GA, Gallanis AF, Gamble LA, McClelland PH, Duemler A, et al. Germline CDH1 Variants and Lifetime Cancer Risk. JAMA. 2024;332(9):722-9.

[48] Kobayashi H, Ohno S, Sasaki Y, Matsuura M. Hereditary breast and ovarian cancer susceptibility genes (review). Oncol Rep. 2013;30(3):1019-29.

[49] Greenhough LA, Liang CC, Belan O, Kunzelmann S, Maslen S, Rodrigo-Brenni MC, et al. Structure and function of the RAD51B-RAD51C-RAD51D-XRCC2 tumour suppressor. Nature. 2023;619(7970):650-7.

[50] Apostolou P, Fostira F. Hereditary breast cancer: the era of new susceptibility genes. Biomed Res Int. 2013;2013:747318.

[51] Nikitin AG, Chudakova DA, Enikeev RF, Sakaeva D, Druzhkov M, Shigapova LH, et al. Lynch Syndrome Germline Mutations in Breast Cancer: Next Generation Sequencing Case-Control Study of 1,263 Participants. Front Oncol. 2020;10:666.

[52] Sy SM, Huen MS, Chen J. PALB2 is an integral component of the BRCA complex required for homologous recombination repair. Proc Natl Acad Sci U S A. 2009;106(17):7155-60.

[53] Zhang F, Ma J, Wu J, Ye L, Cai H, Xia B, et al. PALB2 links BRCA1 and BRCA2 in the DNA-damage response. Curr Biol. 2009;19(6):524-9.

[54] Dray E, Etchin J, Wiese C, Saro D, Williams GJ, Hammel M, et al. Enhancement of RAD51 recombinase activity by the tumor suppressor PALB2. Nat Struct Mol Biol. 2010;17(10):1255-9.

[55] Xia B, Dorsman JC, Ameziane N, de Vries Y, Rooimans MA, Sheng Q, et al. Fanconi anemia is associated with a defect in the BRCA2 partner PALB2. Nat Genet. 2007;39(2):159-61.

[56] Rahman N, Seal S, Thompson D, Kelly P, Renwick A, Elliott A, et al. PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. Nat Genet. 2007;39(2):165-7.

[57] Tung N, Battelli C, Allen B, Kaldate R, Bhatnagar S, Bowles K, et al. Frequency of mutations in individuals with breast cancer referred for BRCA1 and BRCA2 testing using next-generation sequencing with a 25-gene panel. Cancer. 2015;121(1):25-33.

[58] Wu S, Zhou J, Zhang K, Chen H, Luo M, Lu Y, et al. Molecular Mechanisms of PALB2 Function and Its Role in Breast Cancer Management. Front Oncol. 2020;10:301.

[59] Moslemi M, Moradi Y, Dehghanbanadaki H, Afkhami H, Khaledi M, Sedighimehr N, et al. The association between ATM variants and risk of breast cancer: a systematic review and meta-analysis. BMC Cancer. 2021;21(1):27.

[60] Falck J, Mailand N, Syljuasen RG, Bartek J, Lukas J. The ATM-Chk2-Cdc25A checkpoint pathway guards against radioresistant DNA synthesis. Nature. 2001;410(6830):842-7.

[61] Swift M, Reitnauer PJ, Morrell D, Chase CL. Breast and other cancers in families with ataxia-telangiectasia. N Engl J Med. 1987;316(21):1289-94.

[62] Economopoulou P, Dimitriadis G, Psyrri A. Beyond BRCA: new hereditary breast cancer susceptibility genes. Cancer Treat Rev. 2015;41(1):1-8.

[63] Decker B, Allen J, Luccarini C, Pooley KA, Shah M, Bolla MK, et al. Rare, protein-truncating variants in ATM, CHEK2 and PALB2, but not XRCC2, are associated with increased breast cancer risks. J Med Genet. 2017;54(11):732-41.

[64] Ratajska M, Antoszewska E, Piskorz A, Brozek I, Borg A, Kusmierek H, et al. Cancer predisposing BARD1 mutations in breast-ovarian cancer families. Breast Cancer Res Treat. 2012;131(1):89-97.

[65] Peduto C, Zanobio M, Nigro V, Perrotta S, Piluso G, Santoro C. Neurofibromatosis Type 1: Pediatric Aspects and Review of Genotype-Phenotype Correlations. Cancers (Basel). 2023;15(4).

[66] Bergoug M, Doudeau M, Godin F, Mosrin C, Vallee B, Benedetti H. Neurofibromin Structure, Functions and Regulation. Cells. 2020;9(11).

[67] Suarez-Kelly LP, Yu L, Kline D, Schneider EB, Agnese DM, Carson WE. Increased breast cancer risk in women with neurofibromatosis type 1: a meta-analysis and systematic review of the literature. Hered Cancer Clin Pract. 2019;17:12.

[68] Yendamuri S, Trapasso F, Calin GA. ARLTS1 - a novel tumor suppressor gene. Cancer Lett. 2008;264(1):11-20.

[69] Akisik E, Yazici H, Dalay N. ARLTS1, MDM2 and RAD51 gene variations are associated with familial breast cancer. Mol Biol Rep. 2011;38(1):343-8.

[70] Xie N, Shu Q, Wang Z, Huang X, Wang Y, Qin B, et al. ARL11 correlates with the immunosuppression and poor prognosis in breast cancer: A comprehensive bioinformatics analysis of ARL family members. PLoS One. 2022;17(11):e0274757.

[71] Kuo SH, Wei MF, Lee YH, Lin JC, Yang WC, Yang SY, et al. MAP3K1 expression is associated with progression and poor prognosis of hormone receptor-positive, HER2-negative early-stage breast cancer. Cell Oncol (Dordr). 2023;46(5):1213-34.

[72] Turbin DA, Cheang MC, Bajdik CD, Gelmon KA, Yorida E, De Luca A, et al. MDM2 protein expression is a negative prognostic marker in breast carcinoma. Mod Pathol. 2006;19(1):69-74.

[73] Haupt S, Vijayakumaran R, Miranda PJ, Burgess A, Lim E, Haupt Y. The role of MDM2 and MDM4 in breast cancer development and prevention. J Mol Cell Biol. 2017;9(1):53-61.

[74] Gao C, Xiao G, Piersigilli A, Gou J, Ogunwobi O, Bargonetti J. Context-dependent roles of MDMX (MDM4) and MDM2 in breast cancer proliferation and circulating tumor cells. Breast Cancer Res. 2019;21(1):5.

[75] Mavaddat N, Antoniou AC, Easton DF, Garcia-Closas M. Genetic susceptibility to breast cancer. Mol Oncol. 2010;4(3):174-91.

[76] Brett JO, Spring LM, Bardia A, Wander SA. ESR1 mutation as an emerging clinical biomarker in metastatic hormone receptor-positive breast cancer. Breast Cancer Res. 2021;23(1):85.

[77] Wang B, Matsuoka S, Ballif BA, Zhang D, Smogorzewska A, Gygi SP, et al. Abraxas and RAP80 form a BRCA1 protein complex required for the DNA damage response. Science. 2007;316(5828):1194-8.

[78] Ikeda Y, Kiyotani K, Yew PY, Kato T, Tamura K, Yap KL, et al. Germline PARP4 mutations in patients with primary thyroid and breast cancers. Endocr Relat Cancer. 2016;23(3):171-9.

[79] Fairoosa P, Witharana C. Gene Mutations in Hereditary Breast Cancer- A Review. European Journal of Medical and Health Sciences. 2020;2(3).

[80] Kenemans P, Verstraeten RA, Verheijen RH. Oncogenic pathways in hereditary and sporadic breast cancer. Maturitas. 2008;61(1-2):141-50.

[81] Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature. 2009;461(7267):1071-8.

[82] Huang R, Zhou PK. DNA damage repair: historical perspectives, mechanistic pathways and clinical translation for targeted cancer therapy. Signal Transduct Target Ther. 2021;6(1):254.

[83] Nguyen L, J WMM, Van Hoeck A, Cuppen E. Pan-cancer landscape of homologous recombination deficiency. Nat Commun. 2020;11(1):5584.

[84] Yang Y, Lian B, Si L, Chi Z, Sheng X, Kong Y, et al. 851P Frequency and clinical significance of homologous recombination deficiency gene mutations in non-cutaneous melanoma. Annals of Oncology. 2022;33:S939.

[85] Vijayan P, Bonilla L. A brief overview of homologous recombination deficiency testing in cancers for the 'Next-Generation' Pathologist. Journal of Pathology of Nepal. 2020;10(2):1760-5.

[86] Moore GM, Powell SN, Higginson DS, Khan AJ. Examining the prevalence of homologous recombination repair defects in ER+ breast cancers. Breast Cancer Res Treat. 2022;192(3):649-53.

[87] Timms KM, Abkevich V, Neff C, Morris B, Potter J, Tran TV, et al. Abstract 1763: Frequency of homologous recombination repair defects across breast cancer subtypes. Cancer Research. 2013;73(8_Supplement):1763-.

[88] Galland L, Roussot N, Desmoulins I, Mayeur D, Kaderbhai C, Ilie S, et al. Clinical Utility of Genomic Tests Evaluating Homologous Recombination Repair Deficiency (HRD) for Treatment Decisions in Early and Metastatic Breast Cancer. Cancers (Basel). 2023;15(4).

[89] Herzog TJ, Vergote I, Gomella LG, Milenkova T, French T, Tonikian R, et al. Testing for homologous recombination repair or homologous recombination deficiency for poly (ADP-ribose) polymerase inhibitors: A current perspective. European Journal of Cancer. 2023;179:136-46.

[90] Eiriz I, Batista MV, Freitas AR, Martins T, Machado C, Braga S, et al. PARP inhibitors in HRD BRCAness breast cancer patients. Journal of Cancer Biology. 2023;4(1):17-28.

[91] Huang Y, Qiu Y, Ding L, Ren S, Jiang Y, Luo J, et al. Somatic mutations in four novel genes contribute to homologous recombination deficiency in breast cancer: a real-world clinical tumor sequencing study. J Pathol Clin Res. 2024;10(2):e12367.

[92] Long M, Liu H, Wu J, Wang S, Liao X, Liu Y, et al. Expression of DNA Helicase Genes Was Correlated with Homologous Recombination Deficiency in Breast Cancer. Comput Math Methods Med. 2022;2022:5508301.

[93] Sobral-Leite M, Salomon I, Opdam M, Kruger DT, Beelen KJ, van der Noort V, et al. Cancer-immune interactions in ER-positive breast cancers: PI3K pathway alterations and tumor-infiltrating lymphocytes. Breast Cancer Research. 2019;21(1):90.

[94] Zhang HP, Jiang RY, Zhu JY, Sun KN, Huang Y, Zhou HH, et al. PI3K/AKT/mTOR signaling pathway: an important driver and therapeutic target in triple-negative breast cancer. Breast Cancer. 2024;31(4):539-51.

[95] Hudson CC, Liu M, Chiang GG, Otterness DM, Loomis DC, Kaper F, et al. Regulation of hypoxia-inducible factor 1alpha expression and function by the mammalian target of rapamycin. Mol Cell Biol. 2002;22(20):7004-14.

[96] Roberts DJ, Miyamoto S. Hexokinase II integrates energy metabolism and cellular protection: Akting on mitochondria and TORCing to autophagy. Cell Death Differ. 2015;22(2):248-57.

[97] Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. Nat Rev Cancer. 2009;9(8):550-62.

[98] Dong C, Wu J, Chen Y, Nie J, Chen C. Activation of PI3K/AKT/mTOR Pathway Causes Drug Resistance in Breast Cancer. Front Pharmacol. 2021;12:628690.

[99] Zeng YA, Nusse R. Wnt proteins are self-renewal factors for mammary stem cells and promote their long-term expansion in culture. Cell Stem Cell. 2010;6(6):568-77.

[100] Robinson KF, Narasipura SD, Wallace J, Ritz EM, Al-Harthi L. beta-Catenin and TCFs/LEF signaling discordantly regulate IL-6 expression in astrocytes. Cell Commun Signal. 2020;18(1):93.

[101] Stewart RA, Ding Z, Jeon US, Goodman LB, Tran JJ, Zientko JP, et al. Wnt target gene activation requires beta-catenin separation into biomolecular condensates. PLoS Biol. 2024;22(9):e3002368.

[102] Clevers H. Wnt/beta-catenin signaling in development and disease. Cell. 2006;127(3):469-80.

[103] Wang Y. Wnt/Planar cell polarity signaling: a new paradigm for cancer therapy. Mol Cancer Ther. 2009;8(8):2103-9.

[104] van Loon K, Huijbers EJM, Griffioen AW. Secreted frizzled-related protein 2: a key player in noncanonical Wnt signaling and tumor angiogenesis. Cancer Metastasis Rev. 2021;40(1):191-203.

[105] Liu W. The intersection between Wnt signaling pathway and cancer metabolism. AIP Conference Proceedings. 2022;2589(1).

[106] Xu X, Zhang M, Xu F, Jiang S. Wnt signaling in breast cancer: biological mechanisms, challenges and opportunities. Mol Cancer. 2020;19(1):165.

[107] Xu J, Prosperi JR, Choudhury N, Olopade OI, Goss KH. beta-Catenin is required for the tumorigenic behavior of triple-negative breast cancer cells. PLoS One. 2015;10(2):e0117097.

[108] Geyer FC, Lacroix-Triki M, Savage K, Arnedos M, Lambros MB, MacKay A, et al. beta-Catenin pathway activation in breast cancer is associated with triple-negative phenotype but not with CTNNB1 mutation. Mod Pathol. 2011;24(2):209-31.

[109] Jeon SM, Lim JS, Park SH, Lee JH. Wnt signaling promotes tumor development in part through phosphofructokinase 1 platelet isoform upregulation. Oncol Rep. 2021;46(5).

[110] Chan XY, Chang KP, Yang CY, Liu CR, Hung CM, Huang CC, et al. Upregulation of ENAH by a PI3K/AKT/beta-catenin cascade promotes oral cancer cell migration and growth via an ITGB5/Src axis. Cell Mol Biol Lett. 2024;29(1):136.

[111] Zhao Z, Song Z, Liao Z, Liu Z, Sun H, Lei B, et al. PKM2 promotes stemness of breast cancer cell by through Wnt/beta-catenin pathway. Tumour Biol. 2016;37(3):4223-34.

[112] Lee SY, Jeon HM, Ju MK, Kim CH, Yoon G, Han SI, et al. Wnt/Snail signaling regulates cytochrome C oxidase and glucose metabolism. Cancer Res. 2012;72(14):3607-17.

[113] Yang K, Wang X, Zhang H, Wang Z, Nan G, Li Y, et al. The evolving roles of canonical WNT signaling in stem cells and tumorigenesis: implications in targeted cancer therapies. Lab Invest. 2016;96(2):116-36.

[114] Chakrabarti R, Wei Y, Hwang J, Hang X, Andres Blanco M, Choudhury A, et al. ΔNp63 promotes stem cell activity in mammary gland development and basal-like breast cancer by enhancing Fzd7 expression and Wnt signalling. Nature Cell Biology. 2014;16(10):1004-15.

[115] Beni FA, Kazemi M, Dianat-Moghadam H, Behjati M. MicroRNAs regulating Wnt signaling pathway in colorectal cancer: biological implications and clinical potentials. Functional & Integrative Genomics. 2022;22(6):1073-88.

[116] Wang M, Pan W, Xu Y, Zhang J, Wan J, Jiang H. Microglia-Mediated Neuroinflammation: A Potential Target for the Treatment of Cardiovascular Diseases. J Inflamm Res. 2022;15:3083-94.

[117] Sharma A, Mir R, Galande S. Epigenetic Regulation of the Wnt/beta-Catenin Signaling Pathway in Cancer. Front Genet. 2021;12:681053.

[118] Bai X, Ni J, Beretov J, Graham P, Li Y. Cancer stem cell in breast cancer therapeutic resistance. Cancer Treat Rev. 2018;69:152-63.

[119] Tse CO, Kim S, Park J. Activation of Wnt signaling pathway by AF1q enriches stem-like population and enhance mammosphere formation of breast cells. Biochem Biophys Res Commun. 2017;484(4):884-9.

[120] Zhang M, Zhang L, Geng A, Li X, Zhou Y, Xu L, et al. CDK14 inhibition reduces mammary stem cell activity and suppresses triple negative breast cancer progression. Cell Reports. 2022;40(11):111331.

[121] Mbongue J, Nicholas D, Torrez T, Kim N-S, Firek A, Langridge W. The Role of Indoleamine 2, 3-Dioxygenase in Immune Suppression and Autoimmunity. Vaccines. 2015;3(3):703-29.

[122] Yu J, Wang Y, Yan F, Zhang P, Li H, Zhao H, et al. Noncanonical NF-κB Activation Mediates STAT3-Stimulated IDO Upregulation in Myeloid-Derived Suppressor Cells in Breast Cancer. The Journal of Immunology. 2014;193(5):2574-86.

[123] Végran F, Boidot R, Michiels C, Sonveaux P, Feron O. Lactate Influx through the Endothelial Cell Monocarboxylate Transporter MCT1 Supports an NF-κB/IL-8 Pathway that Drives Tumor Angiogenesis. Cancer Research. 2011;71(7):2550-60.

[124] Pavitra E, Kancharla J, Gupta VK, Prasad K, Sung JY, Kim J, et al. The role of NF-κB in breast cancer initiation, growth, metastasis, and resistance to chemotherapy. Biomedicine & Pharmacotherapy. 2023;163:114822.

[125] Liu W, Singh SR, Hou SX. JAK-STAT is restrained by Notch to control cell proliferation of the Drosophila intestinal stem cells. J Cell Biochem. 2010;109(5):992-9.

[126] Ranganathan P, Weaver KL, Capobianco AJ. Notch signalling in solid tumours: a little bit of everything but not all the time. Nat Rev Cancer. 2011;11(5):338-51.

[127] Kontomanolis EN, Kalagasidou S, Pouliliou S, Anthoulaki X, Georgiou N, Papamanolis V, et al. The Notch Pathway in Breast Cancer Progression. ScientificWorldJournal. 2018;2018:2415489.

[128] Simoes BM, O'Brien CS, Eyre R, Silva A, Yu L, Sarmiento-Castro A, et al. Anti-estrogen Resistance in Human Breast Tumors Is Driven by JAG1-NOTCH4-Dependent Cancer Stem Cell Activity. Cell Rep. 2015;12(12):1968-77.

[129] Zhou L, Wang D, Sheng D, Xu J, Chen W, Qin Y, et al. NOTCH4 maintains quiescent mesenchymal-like breast cancer stem cells via transcriptionally activating SLUG and GAS1 in triple-negative breast cancer. Theranostics. 2020;10(5):2405-21.

[130] Garcia-Heredia JM, Lucena-Cacace A, Verdugo-Sivianes EM, Perez M, Carnero A. The Cargo Protein MAP17 (PDZK1IP1) Regulates the Cancer Stem Cell Pool Activating the Notch Pathway by Abducting NUMB. Clin Cancer Res. 2017;23(14):3871-83.

[131] Zhang Y, Xu B, Zhang XP. Effects of miRNAs on functions of breast cancer stem cells and treatment of breast cancer. Onco Targets Ther. 2018;11:4263-70.

[132] Zhao D, Mo Y, Li MT, Zou SW, Cheng ZL, Sun YP, et al. NOTCH-induced aldehyde dehydrogenase 1A1 deacetylation promotes breast cancer stem cells. J Clin Invest. 2014;124(12):5453-65.

[133] Cui J, Li P, Liu X, Hu H, Wei W. Abnormal expression of the Notch and Wnt/beta-catenin signaling pathways in stem-like ALDH(hi)CD44(+) cells correlates highly with Ki-67 expression in breast cancer. Oncol Lett. 2015;9(4):1600-6.

[134] Yousefi H, Bahramy A, Zafari N, Delavar MR, Nguyen K, Haghi A, et al. Notch signaling pathway: a comprehensive prognostic and gene expression profile analysis in breast cancer. BMC Cancer. 2022;22(1):1282.

[135] Li L, Tang P, Li S, Qin X, Yang H, Wu C, et al. Notch signaling pathway networks in cancer metastasis: a new target for cancer therapy. Med Oncol. 2017;34(10):180.

[136] Hossain F, Sorrentino C, Ucar DA, Peng Y, Matossian M, Wyczechowska D, et al. Notch Signaling Regulates Mitochondrial Metabolism and NF-kappaB Activity in Triple-Negative Breast Cancer Cells via IKKalpha-Dependent Non-canonical Pathways. Front Oncol. 2018;8:575.

[137] BeLow M, Osipo C. Notch Signaling in Breast Cancer: A Role in Drug Resistance. Cells. 2020;9(10).

[138] Darbeheshti F, Mahdiannasser M, Noroozi Z, Firoozi Z, Mansoori B, Daraei A, et al. Circular RNA-associated ceRNA network involved in HIF-1 signalling in triple-negative breast cancer: circ_0047303 as a potential key regulator. Journal of Cellular and Molecular Medicine. 2021;25(24):11322-32.

[139] Bruick RK, McKnight SL. A conserved family of prolyl-4-hydroxylases that modify HIF. Science. 2001;294(5545):1337-40.

[140] Lando D, Peet DJ, Whelan DA, Gorman JJ, Whitelaw ML. Asparagine Hydroxylation of the HIF Transactivation Domain: A Hypoxic Switch. Science. 2002;295(5556):858-61.

[141] Iwai K, Yamanaka K, Kamura T, Minato N, Conaway RC, Conaway JW, et al. Identification of the von Hippel-lindau tumor-suppressor protein as part of an active E3 ubiquitin ligase complex. Proc Natl Acad Sci U S A. 1999;96(22):12436-41.

[142] Mahon PC, Hirota K, Semenza GL. FIH-1: a novel protein that interacts with HIF-1alpha and VHL to mediate repression of HIF-1 transcriptional activity. Genes Dev. 2001;15(20):2675-86.

[143] Luo S, Jiang Y, Anfu Z, Zhao Y, Wu X, Li M, et al. Targeting hypoxia-inducible factors for breast cancer therapy: A narrative review. Frontiers in Pharmacology. 2022;13.

[144] Liu ZJ, Semenza GL, Zhang HF. Hypoxia-inducible factor 1 and breast cancer metastasis. J Zhejiang Univ Sci B. 2015;16(1):32-43.

[145] Lee P, Chandel NS, Simon MC. Cellular adaptation to hypoxia through hypoxia inducible factors and beyond. Nat Rev Mol Cell Biol. 2020;21(5):268-83.

[146] Cao Q, Mushajiang M, Tang CQ, Ai XQ. Role of hypoxia-inducible factor-1alpha and survivin in breast cancer recurrence and prognosis. Heliyon. 2023;9(3):e14132.

[147] Atlante S, Visintin A, Marini E, Savoia M, Dianzani C, Giorgis M, et al. α-ketoglutarate dehydrogenase inhibition counteracts breast cancer-associated lung metastasis. Cell Death & Disease. 2018;9(7):756.

[148] Ma JH, Qin L, Li X. Role of STAT3 signaling pathway in breast cancer. Cell Commun Signal. 2020;18(1):33.

[149] Yu H, Lee H, Herrmann A, Buettner R, Jove R. Revisiting STAT3 signalling in cancer: new and unexpected biological functions. Nat Rev Cancer. 2014;14(11):736-46.

[150] Yu J, Wang Y, Yan F, Zhang P, Li H, Zhao H, et al. Noncanonical NF-kappaB activation mediates STAT3-stimulated IDO upregulation in myeloid-derived suppressor cells in breast cancer. J Immunol. 2014;193(5):2574-86.

[151] Demaria M, Giorgi C, Lebiedzinska M, Esposito G, D'Angeli L, Bartoli A, et al. A STAT3-mediated metabolic switch is involved in tumour transformation and STAT3 addiction. Aging (Albany NY). 2010;2(11):823-42.

[152] Li YJ, Zhang C, Martincuks A, Herrmann A, Yu H. STAT proteins in cancer: orchestration of metabolism. Nat Rev Cancer. 2023;23(3):115-34.

[153] Wang L, Zhang S, Wang X. The Metabolic Mechanisms of Breast Cancer Metastasis. Front Oncol. 2020;10:602416.

[154] Zhang C, Lin M, Wu R, Wang X, Yang B, Levine AJ, et al. Parkin, a p53 target gene, mediates the role of p53 in glucose metabolism and the Warburg effect. Proc Natl Acad Sci U S A. 2011;108(39):16259-64.

[155] Wang Y, Shi J, Chai K, Ying X, Zhou BP. The Role of Snail in EMT and Tumorigenesis. Curr Cancer Drug Targets. 2013;13(9):963-72.

[156] Funasaka T, Hogan V, Raz A. Phosphoglucose isomerase/autocrine motility factor mediates epithelial and mesenchymal phenotype conversions in breast cancer. Cancer Res. 2009;69(13):5349-56.

[157] Cai F, Xiao H, Sun Y, Wang D, Tang J. Expression of Snail and E-cadherin in Drug-resistant MCF-7/ADM Breast Cancer Cell Strains. J Coll Physicians Surg Pak. 2019;29(3):240-4.

[158] Pavlides S, Whitaker-Menezes D, Castello-Cros R, Flomenberg N, Witkiewicz AK, Frank PG, et al. The reverse Warburg effect: aerobic glycolysis in cancer associated fibroblasts and the tumor stroma. Cell Cycle. 2009;8(23):3984-4001.

[159] Zhang D, Wang H, Yu W, Qiao F, Su X, Xu H. Downregulation of hexokinase 2 improves radiosensitivity of breast cancer. Transl Cancer Res. 2019;8(1):290-7.

[160] Lee JH. Phosphofructokinase 1 Platelet Isoform Enhances VEGF Expression in Part Through HIF-1alpha Up-regulation in Breast Cancer. Anticancer Res. 2023;43(1):75-84.

[161] Malhotra G, Gattani RG, Shinde RK, Gianchandani SG, Nayak K, Salwan A. Significance of Serum Lactate Dehydrogenase as a Prognostic Marker and Outcome Predictor in Patients With Breast Cancer. Cureus. 2024;16(3):e55932.

[162] Wang S, Ma L, Wang Z, He H, Chen H, Duan Z, et al. Lactate Dehydrogenase-A (LDH-A) Preserves Cancer Stemness and Recruitment of Tumor-Associated Macrophages to Promote Breast Cancer Progression. Front Oncol. 2021;11:654452.

[163] Choi J, Kim DH, Jung WH, Koo JS. Metabolic interaction between cancer cells and stromal cells according to breast cancer molecular subtype. Breast Cancer Res. 2013;15(5):R78.

[164] Gomes AS, Ramos H, Soares J, Saraiva L. p53 and glucose metabolism: an orchestra to be directed in cancer therapy. Pharmacol Res. 2018;131:75-86.

[165] Wellberg EA, Johnson S, Finlay-Schultz J, Lewis AS, Terrell KL, Sartorius CA, et al. The glucose transporter GLUT1 is required for ErbB2-induced mammary tumorigenesis. Breast Cancer Res. 2016;18(1):131.

[166] Krzeslak A, Wojcik-Krowiranda K, Forma E, Jozwiak P, Romanowicz H, Bienkiewicz A, et al. Expression of GLUT1 and GLUT3 glucose transporters in endometrial and breast cancers. Pathol Oncol Res. 2012;18(3):721-8.

[167] Sun X, Wang M, Wang M, Yu X, Guo J, Sun T, et al. Metabolic Reprogramming in Triple-Negative Breast Cancer. Front Oncol. 2020;10:428.

[168] Benito A, Polat IH, Noe V, Ciudad CJ, Marin S, Cascante M. Glucose-6-phosphate dehydrogenase and transketolase modulate breast cancer cell metabolic reprogramming and correlate with poor patient outcome. Oncotarget. 2017;8(63):106693-706.

[169] Choi J, Kim ES, Koo JS. Expression of Pentose Phosphate Pathway-Related Proteins in Breast Cancer. Dis Markers. 2018;2018:9369358.

[170] Akella NM, Ciraku L, Reginato MJ. Fueling the fire: emerging role of the hexosamine biosynthetic pathway in cancer. BMC Biol. 2019;17(1):52.

[171] Ferrer CM, Lynch TP, Sodi VL, Falcone JN, Schwab LP, Peacock DL, et al. O-GlcNAcylation regulates cancer metabolism and survival stress signaling via regulation of the HIF-1 pathway. Mol Cell. 2014;54(5):820-31.

[172] Blucher C, Stadler SC. Obesity and Breast Cancer: Current Insights on the Role of Fatty Acids and Lipid Metabolism in Promoting Breast Cancer Growth and Progression. Front Endocrinol (Lausanne). 2017;8:293.

[173] Antalis CJ, Arnold T, Rasool T, Lee B, Buhman KK, Siddiqui RA. High ACAT1 expression in estrogen receptor negative basal-like breast cancer cells is associated with LDL-induced proliferation. Breast Cancer Res Treat. 2010;122(3):661-70.

[174] Magnard C, Bachelier R, Vincent A, Jaquinod M, Kieffer S, Lenoir GM, et al. BRCA1 interacts with acetyl-CoA carboxylase through its tandem of BRCT domains. Oncogene. 2002;21(44):6729-39.

[175] Broadfield LA, Pane AA, Talebi A, Swinnen JV, Fendt SM. Lipid metabolism in cancer: New perspectives and emerging mechanisms. Dev Cell. 2021;56(10):1363-93.

[176] Nomura DK, Long JZ, Niessen S, Hoover HS, Ng SW, Cravatt BF. Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis. Cell. 2010;140(1):49-61.

[177] Jin Q, Yuan LX, Boulbes D, Baek JM, Wang YN, Gomez-Cabello D, et al. Fatty acid synthase phosphorylation: a novel therapeutic target in HER2-overexpressing breast cancer cells. Breast Cancer Res. 2010;12(6):R96.

[178] Kim S, Lee Y, Koo JS. Differential expression of lipid metabolism-related proteins in different breast cancer subtypes. PLoS One. 2015;10(3):e0119473.

[179] Kleinfeld AM, Okada C. Free fatty acid release from human breast cancer tissue inhibits cytotoxic T-lymphocyte-mediated killing. J Lipid Res. 2005;46(9):1983-90.

[180] Li S, Zeng H, Fan J, Wang F, Xu C, Li Y, et al. Glutamine metabolism in breast cancer and possible therapeutic targets. Biochem Pharmacol. 2023;210:115464.

[181] Kim S, Kim DH, Jung WH, Koo JS. Expression of glutamine metabolism-related proteins according to molecular subtype of breast cancer. Endocr Relat Cancer. 2013;20(3):339-48.

[182] Kung HN, Marks JR, Chi JT. Glutamine synthetase is a genetic determinant of cell type-specific glutamine independence in breast epithelia. PLoS Genet. 2011;7(8):e1002229.

[183] Lee CM, Hwang Y, Kim M, Park YC, Kim H, Fang S. PHGDH: a novel therapeutic target in cancer. Exp Mol Med. 2024;56(7):1513-22.

[184] Possemato R, Marks KM, Shaul YD, Pacold ME, Kim D, Birsoy K, et al. Functional genomics reveal that the serine synthesis pathway is essential in breast cancer. Nature. 2011;476(7360):346-50.

[185] Bodineau C, Tome M, Courtois S, Costa ASH, Sciacovelli M, Rousseau B, et al. Two parallel pathways connect glutamine metabolism and mTORC1 activity to regulate glutamoptosis. Nat Commun. 2021;12(1):4814.

[186] Naimi A, Mohammed RN, Raji A, Chupradit S, Yumashev AV, Suksatan W, et al. Tumor immunotherapies by immune checkpoint inhibitors (ICIs); the pros and cons. Cell Commun Signal. 2022;20(1):44.

[187] Thomas R, Al-Khadairi G, Decock J. Immune Checkpoint Inhibitors in Triple Negative Breast Cancer Treatment: Promising Future Prospects. Front Oncol. 2020;10:600573.

[188] Bense RD, Sotiriou C, Piccart-Gebhart MJ, Haanen J, van Vugt M, de Vries EGE, et al. Relevance of Tumor-Infiltrating Immune Cell Composition and Functionality for Disease Outcome in Breast Cancer. J Natl Cancer Inst. 2017;109(1).

[189] Heimes AS, Schmidt M. Atezolizumab for the treatment of triple-negative breast cancer. Expert Opin Investig Drugs. 2019;28(1):1-5.

[190] Santa-Maria CA, Kato T, Park JH, Kiyotani K, Rademaker A, Shah AN, et al. A pilot study of durvalumab and tremelimumab and immunogenomic dynamics in metastatic breast cancer. Oncotarget. 2018;9(27):18985-96.

acta medica

ORIGINAL ARTICLE

Assessment of the relationship between serum uric acid levels and oxidative stress markers in patients with uncomplicated type 2 diabetes mellitus

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ABSTRACT

Objective: Type 2 Diabetes Mellitus is a worldwide health issue characterized by hyperglycemia due to defects in insulin secretion, insulin action, or both, and is associated with significant negative health outcomes. Oxidative stress plays a crucial role in the onset, progression, and complications of this disorder. Elevated serum uric acid level is an independent predictor of vascular complications in diabetic patients, and hyperuricemia may contribute to oxidative stress. The objective of this study is to examine the correlation between increased serum uric acid levels and the markers of oxidative stress, including ischemia-modified albumin (IMA), total oxidant status (TOC), and total antioxidant capacity (TAC), in type 2 diabetic patients who don't have vascular complications.

Materials and Methods: A total of 73 individuals were enrolled in the study including 20 type 2 diabetic patients with high serum uric acid levels (> 6.5 mg/dl), 21 type 2 diabetic patients with normal serum uric acid levels (< 6.5 mg/dl), and 32 healthy individuals. Ischemia-modified albumin, total oxidant status, and total antioxidant status levels were compared between patient groups and the control group

Results: As a result of this study, there was no significant association between serum uric acid levels and ischemia-modified albumin, total oxidant status, and total antioxidant status levels in type 2 diabetic patients.

Conclusions: This study concluded that high serum uric acid levels do not directly affect oxidative stress in type 2 diabetic patients without vascular complications.

Keywords: diabetes mellitus, uric acid, ischemia-modified albumin, total oxidant status, total antioxidant status.

INTRODUCTION

Increased advanced glycosylation end products due to activation of the polyol; hexosamine pathway and protein kinase c activation induced by hyperglycemia is the main pathophysiological mechanism responsible for the complications of type 2 diabetes mellitus (type 2 DM) [1]. In this way, oxidative stress increases, tissue damage occurs and chronic complications of diabetes emerge [2,3].

On the other hand, high serum uric acid levels are also known to be an independent risk factor for vascular complications and mortality in type 2 DM [4]. Each 1 mg/dL increase in serum uric acid level has been reported to increase the risk of developing type 2 DM by 20% [5].

Even though uric acid is considered an antioxidant molecule, it is also known that antioxidant molecules play a role as prooxidants in certain situations [6]. At the beginning of the atherosclerotic process, uric acid acts as an antioxidant molecule. In contrast, when the atherosclerotic process progresses and serum uric acid levels increase, uric acid acts as an oxidant molecule [7]. During the production of uric acid, xanthine formation from hypoxanthine, uric acid formation from xanthine, and one superoxide radical is formed in both steps.

In the presence of oxidative stress, hypoxia, acidosis, and ischemia, a change occurs in the N-terminal region of albumin, which is the binding site for divalent metals such as copper, nickel, and cobalt. This modified form of albumin is known as ischemia-modified albumin (IMA). The metal binding rate of the N-terminal part decreases due to this molecular change. Studies are showing that IMA levels are high in patients with type 2 diabetes, which may be related to a chronic hypoxic state triggered by hyperglycemia and oxidative stress, endothelial dysfunction, and chronic inflammation [8,9].

The level of oxidative stress in the whole body is shown by total oxidant capacity (TOC). For this reason, it is more valuable than assessing oxidant radicals separately [10,11]. In the human body, oxidant and antioxidant systems are normally in balance. In the presence of oxidative stress, the oxidant-antioxidant balance in the body is known to shift towards the oxidant side. Increased oxidative stress is recognized as a contributor to the pathogenesis of insulin resistance and type 2 DM. In the literature, studies indicate that total antioxidant capacity (TAC) decreases in diabetic patients in direct correlation with this mechanism [10]. It is also known that TAC levels are low in prediabetic patients [11]. In diabetic patients with complications, the TAC level is lower than in uncomplicated diabetic patients [12].

This study aims to evaluate the potential relationship between serum uric acid levels and markers of oxidative status, specifically IMA, TOC, and TAC, in patients with type 2 DM without microvascular and macrovascular complications.

MATERIALS and METHODS

The study was designed as a single-center, case-controlled cohort study. A total of 73 participants, including 21 patients with type 2 DM with normal uric acid levels, 20 patients with type 2 DM with increased uric acid levels, and 32 healthy individuals between the ages of 30-70 years, who applied to the Internal Medicine outpatient clinic of Ufuk University Faculty of Medicine Dr. Rıdvan Ege Hospital, were included in the study.

Patients' demographic characteristics, anamnesis, and physical examination findings were recorded. Fasting plasma glucose (FPG), blood urea nitrogen (BUN), creatinine, uric acid, complete blood count, lipid profile, HbA1c, fasting insulin level, C- Reactive Protein (CRP), albumin/creatinine in spot urine were measured. Additionally, a 12-lead electrocardiogram (ECG) was performed for each subject, and ophthalmologic evaluations were carried out.

Anthropometric measurements of the participants were recorded. Body mass index (BMI) was calculated using the formula weight/(height)^2 (kg/m^2). Type 2 DM was diagnosed according to the American Diabetes Association (ADA) criteria [13]. Patients previously diagnosed with diabetes and receiving diabetes treatment were also included in the study.

Patients were evaluated for microvascular and macrovascular complications. Nephropathy was assessed by measuring the albumin-to-

creatinine ratio in spot urine samples. A spot urine albumin/creatinine ratio < 30 mg/day was considered an absence of diabetic nephropathy. The glomerular filtration rate was calculated using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) formula based on serum creatinine measurements [14]. For diabetic retinopathy, past ophthalmologic examinations were reviewed from the patient files. Neuropathy was assessed through patient history, clinical findings, and neurological examination. Clinical findings, ECG data, cardiologic examination, and test results of the patients were evaluated after questioning about cardiovascular risk factors for the presence of coronary heart disease (CHD). Diabetic foot, peripheral arterial disease (PAD), and cerebrovascular accident (CVA) were evaluated by anamnesis, clinical findings, and patient follow-up notes.

Patients were divided into two groups based on their serum uric acid levels: those with levels of 6.5 mg/dL or above were classified as the high uric acid group, while those with levels below 6.5 mg/dL were classified as the normal uric acid group. Control group individuals were selected from hospital employees and patients' relatives who applied to our department and agreed to participate in the study, who had no disease, and whose routine laboratory values were within the normal range. The cut-off value for IMA was determined by measuring IMA levels in the healthy control group, and IMA levels were assessed in comparison to this cut-off value. Ten milliliters (ml) of venous blood samples were obtained from patients with patients with type 2 DM for IMA, TOC, and TAC levels at the Endocrinology and Metabolism Department of Ufuk University Dr. Rıdvan Ege Hospital. The serum of 10 ml of blood was separated and stored at -80°C until the day of the study. On the day of the study, all samples were brought to room temperature, and the study was completed on the same day and the results were evaluated.

Hypertension, known history of CHD, decompensated heart failure, chronic liver, and kidney disease, diabetic nephropathy, retinopathy, neuropathy, history of transient ischemic attack or ischemic CVA, acute and chronic infection (those with significantly increased CRP levels and those with laboratory and physical examination findings in favor of infection), vitamin and antioxidant use, history of PAH, any diagnosed malignant disease and paraproteinemia, pregnant and lactating females, diuretic drug users, those aged <30 and >70 years, and those who refused to give informed consent were excluded.

Biochemical evaluation methods

Plasma fasting glucose levels were determined by Hexokinase/G-6-PDH (Glucose 6 Phosphate Dehydrogenase) method and serum total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride (TG), BUN, and uric acid levels were determined colorimetrically. Fasting insulin levels were determined by chemiluminescent microparticle immunoassay (CMIA). HbA1c level was measured by high-performance liquid chromatography (HPLC). Serum creatinine was evaluated by the kinetic alkaline picrate method. CRP was measured by the immunoturbidimetric method. In spot urine, albumin was measured turbidimetrically, and creatinine was measured by kinetic alkaline picrate technology.

Ischemia-modified albumin, the complex formed by cobalt (II) not bound to albumin with dithioerythritol was measured by colorimetric method at 470 nm with a spectrophotometer. Results were given in Absorbance unit (ABSU). TOC was measured with a spectrophotometer at 530 nm and the results were calculated as micromol/L. TAC was measured with a spectrophotometer at 660 nm and the results were calculated as mmol/L.

Statistical analysis

The IBM SPSS Statistics 20.0 software was used for statistical analysis of the data. Continuous data were presented as mean ± standard deviation, and median (minimum-maximum value); discrete data were presented as frequency and percentage. Shapiro Wilk's test was used to evaluate whether there was a normal distribution among the variables. For normally distributed groups, comparisons were made using the t-test for independent samples, whereas the Mann-Whitney U test was used for non-normally distributed groups. The Chi-square test was used to compare the percentage data between groups. The Kruskal-Wallis test was used to compare IMA levels among three groups of diabetic patients. The correlations between the variables were analyzed with Spearman's correlation coefficient. In the study, $p<0.05$ was considered statistically significant.

Ethical approval

This study (21012015-1) was approved in terms of medical ethics by the Ufuk University Faculty of Medicine Dr. Rıdvan Ege Hospital Non-Interventional Clinical Research Ethics Committee and was conducted by the Declaration of Helsinki. The informed consent form was obtained from each patient participating in the study.

RESULTS

Comparison of diabetic groups with high and normal uric acid levels

A total of 41 subjects, including 20 (10 F/10 M) diabetic patients with high uric acid levels and 21 (10 F/11 M) diabetic patients with normal uric acid levels, with a mean age of 60.9±7.1 years and BMI of 27.9±2.9 kg/m^2, were included in the study. Detailed comparisons of the groups concerning demographic, clinical, and biochemical variables are shown in Table 1. It was found that patients were homogeneously distributed in terms of age, gender, and BMI in both groups. The BUN level of diabetic patients in the group with high uric acid levels was statistically significantly higher than that of diabetic patients with normal uric acid levels (p=0.034). No statistically significant difference was found between other biochemical parameters between both groups.

The correlation between variables was evaluated using Spearman's correlation coefficient for a sample of 41 patients with type 2 DM, a statistically significant correlation was found between the HbA1c levels and BMI (p<0.001, r=0.71). A significant correlation was also found between HbA1c - CRP (p=0.041, r=0.54) and BMI - CRP (p=0.046, r=0.48) levels. A statistically significant negative correlation was found between HDL-HbA1c (p=0.025, r=-0.57).

Ischemia-modified albumin levels of diabetics and healthy individuals with normal and high uric acid levels

IMA levels in 41 diabetic patients (21 patients with normal uric acid/20 patients with increased uric acid) and 32 healthy controls were evaluated. As shown in Table 2, a detailed comparison of the groups based on IMA levels revealed no significant differences (p=0.332).

Table 1. Comparison of diabetic groups with high and normal uric acid levels

	Diabetics with high uric acid (n:20)	Diabetics with normal uric acid (n:21)	P value
Age (years)	61.3±7.1	60.5±7.2	0.715
F/M (n, %)	10/10 (50.0/50.0)	10/11 (48.8/51.2)	0.896
BMI (kg/m^2)	27.3±2.5	28.5±3.2	0.191
Uric acid (mg/dL)	7.7 (9/6.8) ‡	4.9 (6.4/2.9) ‡	< 0.001
FPG (mg/dL)	149 (253/98) ‡	195.1 (358/104) ‡	0.213
HbA1c (%)	7.16±1.34	7.65±1.97	0.359
Total cholesterol (mg/dL)	204.9±62.5	194±43.6	0.518
LDL (mg/dL)	123.7±43.9	115.4±35.8	0.514
HDL (mg/dL)	37.8±7	42.4±11.9	0.142
Triglycerides (mg/dL)	200.5±115.7	191±118	0.817
BUN (mg/dL)	18.2±4.8	15±4.6	0.034
Creatinine (mg/dL)	0.96±0.18	0.86±0.17	0.076
Spot urine alb/cre (mg/day)	14.3±7.9	13.9±6.4	0.867
Hb (g/dL)	14.9±1.8	15.5±1.5	0.566
CRP (mg/L)	4±2.6	3.2±2.3	0.317
IMA (ABSU)	0.38±0.16	0.40±0.16	0.846

*F/M: Female/Male, BMI: Body mass index, FPG: Fasting plasma glucose, HbA1c: Glycated hemoglobin, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, BUN: Blood urea nitrogen, Hb: Hemoglobin, Alb/cre: Albumin/creatinine ratio, CRP: C-Reactive protein, IMA: Ischemia modified albumin, ±: Standard deviation, ‡: Min. – Max. values

Table 2. Ischemia-modified albumin (IMA) levels of diabetics and healthy individuals with normal and high uric acid levels

	Diabetics with high uric acid (n:20)	Diabetics with normal uric acid (n:21)	Healthy individuals (n:32)	P value
Age (years)	61.3±7.1	60.5±7.2	68±7	
F/M (n, %)	10/10 (50.0/50.0)	10/11 (48.8/51.2)	12/20 (37.5/62.5)	
IMA (ABSU)	0.38±0.16	0.40±0.16	0.37±0.08	(p=0.332)

* F/M: Female/Male, IMA: Ischemia modified albumin, ABSU: absorbance-unit

Comparison of TOC and TAC levels in diabetic groups with high and normal uric acid levels

In all diabetic patients, the mean TOC level was 46.2±45 µmol/L, which was higher than the reference values as expected. Comparing the diabetic groups among themselves, the mean TOC level in the high uric acid group was higher than the TOC level in the normal uric acid group (49.5±45.5 µmol/L, 43±45.4 µmol/L respectively). However, this height is not statistically significant (p=0.655). A mean TAC value of 3.0±0.5 mmol/L was observed in all diabetic patients, which was higher than the reference values. A comparison of diabetic groups according to TAC levels revealed no significant relationship between those with high uric acid levels and those with normal uric acid levels (3.1±0.4 mmol/L, 3.0±0.5 mmol/L respectively) (p=0.461).

DISCUSSION

Hyperuricemia has been associated with hypertension, atherosclerotic cardiovascular disease, chronic kidney disease, and metabolic syndrome [4,15,16].

Metabolic abnormalities in type 2 DM are thought to alter uric acid metabolism, prompting numerous studies to explore this correlation. Animal studies suggest that uric acid may impair insulin resistance by decreasing nitric oxide bioavailability, potentially contributing to diabetes development [17].

However, whether serum uric acid is an independent risk factor for type 2 DM remains unclear. Some studies link serum uric acid levels with carotid atherosclerosis in type 2 DM patients, while others report no significant correlation [16,18]. A Mendelian randomized study by Sluijs et al., found no relationship between serum uric acid levels and diabetes risk, nor did uric acid-lowering treatments affect diabetes development [19]. Nevertheless, other research consistently demonstrates a linear relationship between high uric acid levels and diabetes complications. A meta-analysis by Xu et al., reported that a 1 mg/dl increase in serum uric acid levels raises the risk of vascular complications by 18% and mortality by 9% [4]. Serum uric acid is also considered a risk factor for diabetic nephropathy and is associated with various diabetes complications, suggesting that it should be closely monitored in patients with type 2 DM [20].

This study evaluated the impact of elevated uric acid on IMA, TOC, and TAC levels which are used as indicators of oxidative stress in individuals with type 2 DM. To evaluate the independent effect of uric acid, we excluded patients with type 2 DM who had developed either microvascular or macrovascular complications. Considering the evidence from studies showing a positive relationship between HbA1c levels and uric acid levels [20,21], we formed two groups with statistically similar HbA1c values in our study. Based on the evidence from the literature indicating a positive association between BMI, obesity, and uric acid [22,23], we established groups with similar BMI levels.

To ensure accurate efficacy evaluation and mitigate the confounding effect of uric acid, we excluded patients with elevated CRP levels. While the study included patients with normal CRP levels, our analysis of CRP levels across all groups identified a significant correlation between CRP levels and BMI-HbA1c, as reported in the literature. Gender is another important issue when evaluating uric acid levels [24]. It is higher in males than in females. A possible explanation is that increased renal clearance of urate in females, compared to males, may be attributed to higher estrogen levels [16]. In females, the renal proximal tubular urate transporter is inhibited by estrogenic compounds. Consequently, uric acid levels and pools are typically lower in females than in males until menopause. This

difference is evidenced by the increased incidence of gout observed in males over the age of 30 and in females over the age of 50. To control for the effects of age and gender in our study, we matched the groups to ensure no significant differences in age and gender distribution.

Since studies are showing that uric acid plays a pro-oxidant role when the uric acid level exceeds > 6.5 - 7 mg/dL in males and > 6 mg/dL in females [6,22], we classified type 2 DM patients with uric acid levels> 6.5 mg/dL as a uric acid high group.

This study demonstrated that IMA, TOC, and TAC levels did not differ significantly between patients with type 2 DM who had elevated serum uric acid levels and those with normal uric acid levels.

The pioneering study by Piwowar et al., on IMA levels in patients with type 2 DM, demonstrated elevated IMA levels compared to healthy individuals [25]. Their research identified a significant correlation between IMA levels and HbA1c while showing a weak correlation between IMA levels, blood pressure, and LDL. Piwowar et al., were the first to report that elevated IMA levels in diabetic patients may originate from non-cardiac sources, highlighting that chronic hypoxia-induced by hyperglycemia and oxidative stress can modify the albumin molecule in the plasma of these patients [25]. El-Eshmawy et al. also observed a significant increase in IMA levels in prediabetic individuals compared to healthy controls [26].

Although our study showed higher IMA levels in type 2 DM patients relative to the control group, this difference was not statistically significant. This result is supported by the findings of Dahiya et al., who similarly reported no significant difference in IMA levels between 60 newly diagnosed type 2 DM patients without vascular complications and 30 healthy controls [27]. In line with the results of our study, the study by Ma et al., also reported no significant difference in IMA levels between individuals with type 2 DM without peripheral arterial disease (PAD) and the healthy control group. [28]. Although ischemia-modified albumin is a proven cardiac marker, its role in type 2 DM without vascular complications has not yet been reported [27].

When comparing the results of existing studies with our findings, it can be concluded that IMA levels are significantly elevated in patients with type 2 DM who have complications compared to healthy individuals; however, this difference is not observed in patients with type 2 DM who do not have complications. This discrepancy may be attributed to the fact that oxidative stress, ischemia, vascular endothelial damage, and chronic hypoxia are significantly more pronounced in patients with type 2 DM who have complications compared to those without complications, potentially leading to a significant increase in IMA levels. Besides, in most of the studies that found high levels of IMA in patients with type 2 DM, burayı high sensitive C-reactive protein (hs-CRP) level and IMA were found to be correlated. Kaefer et al., reported that hs-CRP levels were also significantly higher in diabetic patients with high IMA levels [29], but the patient group in our study was selected from patients with normal CRP levels. This may be a factor in the fact that the IMA level was not found to be significantly high. IMA was mainly associated with CRP, a marker of inflammation, rather than diabetes itself in uncomplicated diabetic patients, as in the studies mentioned above [29]. In our study, the high uric acid level did not affect this condition.

According to the results of this study, we found that the mean TOC level was higher than the reference values in all patients with type 2 DM. Numerous studies have indicated that TOC levels are elevated in patients with type 2 DM [30,31]. The findings of our study are consistent with these observations. In the comparison of the study groups with each other, although the TOC level was higher in the group with higher uric acid, this increase was not significant.

In this study, TAC levels were higher than reference values across all patients with type 2 DM, which reflects a contrast with diverse findings in existing literature. This observation is consistent with the study by Savu et al., where increased TAC levels were reported in type 2 DM patients without vascular complications [31]. The body tries to keep the oxidant/antioxidant system in balance. Oxidative stress induces an increase in the activity of antioxidant mechanisms. However, once oxidative stress reaches a threshold level, these antioxidant defenses become inadequate and depleted, leading to a decline in TAC levels [19]. Considering the study results, it is suggested that since the study group consisted of type 2 DM patients without

vascular complications, the level of oxidative stress was likely manageable by antioxidant mechanisms, which could explain the high TAC levels recorded. The comparison of TAC levels across different serum uric acid levels did not reveal any significant differences. When the results related to TOC-TAC were evaluated together, the effect of uric acid level alone on oxidant/antioxidant balance in patients with type 2 DM without vascular complications was not strong enough to make a significant difference. However, the fact that both systems are active indicates that uncomplicated diabetic individuals still have adequate antioxidant capacity to respond to the oxidative response.

The study's strengths lie in its focus on patients with type 2 DM without microvascular or macrovascular complications, offering insights into the early stages of the disease and the role of uric acid in oxidative stress. Additionally, including diabetic patients with varying uric acid levels and healthy controls, it establishes a comparative framework for understanding oxidative markers.

Also, this study has several limitations to consider. Firstly, the small sample size (41 diabetic patients and 32 healthy controls) limits the statistical power to detect significant differences or subtle correlations. Secondly, as a cross-sectional study, it only provides a snapshot of serum uric acid levels and oxidative markers, restricting the ability to infer causal relationships or long-term effects in type 2 DM. Lastly, being a single-center study is also a major limitation.

In conclusion, we did not find a direct correlation between increased serum uric acid level alone and IMA - TOC - TAC in our study. These results imply that increased serum uric acid levels alone do not influence oxidative stress in patients with uncomplicated type 2 diabetes. The study's findings demonstrate that uncomplicated diabetic patients can augment their TAC capacity to sustain antioxidant defenses. Thus, uric acid should not be considered a marker of oxidative stress or a protective factor against oxidative stress in individuals with uncomplicated type 2 DM. Nevertheless, future research should aim to provide a more comprehensive understanding of how serum uric acid levels influence the oxidant/antioxidant balance in patients with type 2 DM. Long-term studies are also needed to follow diabetic patients without complications and to explore the relationships between uric acid, IMA, TOC, and TAC as complications progress.

Author contribution

Study conception and design: EKK, TC, and IE; data collection: EKK; analysis and interpretation of results: EKK, IE; draft manuscript preparation: EKK, IE. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Ethics Committee of Ufuk University Faculty of Medicine Dr. Rıdvan Ege Hospital (Protocol no: 21012015-1).

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] Lee AY, Chung SS. Contributions of polyol pathway to oxidative stress in diabetic cataract. FASEB J 1999;13(1):23-30. https://doi.org/10.1096/fasebj.13.1.23

[2] Brownlee M. The pathobiology of diabetic complications: A unifying mechanism. Diabetes 2005;54(6):1615-25. https://doi.org/10.2337/diabetes.54.6.1615

[3] Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414(6865):813-20. https://doi.org/10.1038/414813a

[4] Xu Y, Zhu J, Gao L, et al. Hyperuricemia as an independent predictor of vascular complications and mortality in type 2 diabetes patients: A meta-analysis. PLoS One 2013;8(10):e78206. https://doi.org/10.1371/journal.pone.0078206

[5] Bhole V, Choi JWJ, Kim SW, de Vera M, Choi H. Serum uric acid levels and the risk of type 2 diabetes: A prospective study. Am J Med 2010;123(10):957-61. https://doi.org/10.1016/j.amjmed.2010.03.027

[6] Patterson RA, Horsley ETM, Leake DS. Prooxidant and antioxidant properties of human serum ultrafiltrates toward LDL: Important role of uric acid. J Lipid Res 2003;44(3):512-21. https://doi.org/10.1194/jlr.M200407-JLR200

[7] Naghavi M, John R, Naguib S, et al. pH Heterogeneity of human and rabbit atherosclerotic plaques; A new insight into detection of vulnerable plaque. Atherosclerosis 2002;164(1):27-35. https://doi.org/10.1016/s0021-9150(02)00018-7

[8] Ahmad A, Manjrekar P, Yadav C, Agarwal A, Srikantiah RM, Hegde A. Evaluation of Ischemia-Modified Albumin, Malondialdehyde, and Advanced Oxidative Protein Products as Markers of Vascular Injury in Diabetic Nephropathy. Biomark Insights 2016;11:63-8. https://doi.org/10.4137/BMI.S39053

[9] Carter DC, Ho JX. Structure of serum albumin. Adv Protein Chem 1994;45:153-203. https://doi.org/10.1016/s0065-3233(08)60640-3

[10] Rani AJ, Mythili SV. Study on total antioxidant status in relation to oxidative stress in type 2 diabetes mellitus. J Clin Diagn Res 2014;8(3):108-10. https://doi.org/10.7860/JCDR/2014/7603.4121

[11] Song F, Jia W, Yao Y, et al. Oxidative stress, antioxidant status and DNA damage in patients with impaired glucose regulation and newly diagnosed Type 2 diabetes. Clin Sci (Lond) 2007;112(12):599-606. https://doi.org/10.1042/CS20060323

[12] Opara EC, Abdel-Rahman E, Soliman S, et al. Depletion of total antioxidant capacity in type 2 diabetes. Metabolism 1999;48(11):1414-7. https://doi.org/10.1016/s0026-0495(99)90152-x

[13] American Diabetes Association Professional Practice Committee . 2. Diagnosis and Classification of Diabetes: Standards of Care in Diabetes-2024. Diabetes Care 2024;47(Suppl 1):S20-42. https://doi.org/10.2337/dc24-S002

[14] Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150(9):604-12. https://doi.org/10.7326/0003-4819-150-9-200905050-00006

[15] Forman JP, Choi H, Curhan GC. Plasma uric acid level and risk for incident hypertension among men. J Am Soc Nephrol 2007;18(1):287-92. https://doi.org/10.1681/ASN.2006080865

[16] Venishetty S, Bhat R, Rajagopal KV. Serum Uric Acid Levels in Type 2 Diabetes Mellitus: Is There a Linear Relationship with Severity of Carotid Atherosclerosis?. Indian J Endocrinol Metab 2018;22(5):678-82. https://doi.org/10.4103/ijem.IJEM_641_17

[17] Khosla UM, Zharikov S, Finch JL, et al. Hyperuricemia induces endothelial dysfunction. Kidney Int 2005;67(5):1739-42. https://doi.org/10.1111/j.1523-1755.2005.00273.x

[18] Kaiser N, Sasson S, Feener EP, et al. Differential regulation of glucose transport and transporters by glucose in vascular endothelial and smooth muscle cells. Diabetes 1993;42(1):80-9. https://doi.org/10.2337/diab.42.1.80

[19] Sluijs I, Holmes MV, van der Schouw YT, et al. A Mendelian Randomization Study of Circulating Uric Acid and Type 2 Diabetes. Diabetes 2015;64(8):3028-36. https://doi.org/10.2337/db14-0742

[20] Chuengsamarn S, Rattanamongkolgul S, Jirawatnotai S. Association between serum uric acid level and microalbuminuria to chronic vascular complications in Thai patients with type 2 diabetes. J Diabetes Complications 2014;28(2):124-9. https://doi.org/10.1016/j.jdiacomp.2013.12.002

[21] Gill A, Kukreja S, Malhotra N, Chhabra N. Correlation of the serum insulin and the serum uric Acid levels with the glycated haemoglobin levels in the patients of type 2 diabetes mellitus. J Clin Diagn Res 2013;7(7):1295-7. https://doi.org/10.7860/JCDR/2013/6017.3121

[22] Fabbrini E, Serafini M, Colic Baric I, Hazen SL, Klein S. Effect of plasma uric acid on antioxidant capacity, oxidative stress, and insulin sensitivity in obese subjects. Diabetes 2014;63(3):976-81. https://doi.org/10.2337/db13-1396

[23] Johnson RJ, Nakagawa T, Sanchez-Lozada LG, et al. Sugar, uric acid, and the etiology of diabetes and obesity. Diabetes 2013;62(10):3307-15. https://doi.org/10.2337/db12-1814

[24] Kashyap AS, Kashyap S. Hormone replacement therapy and serum uric acid. Lancet 1999;354(9190):1643-4. https://doi.org/10.1016/s0140-6736(05)77128-2

[25] Piwowar A, Knapik-Kordecka M, Warwas M. Ischemia-modified albumin level in type 2 diabetes mellitus - Preliminary report. Dis Markers 2008;24(6):311-7. https://doi.org/10.1155/2008/784313

[26] El-Eshmawy MM, Gad DF, El-Baiomy AA. Elevated Serum Levels of Ischemia Modified Albumin and Malondialdehyde are Related to Atherogenic Index of Plasma in a Cohort of Prediabetes. Endocr Metab Immune Disord Drug Targets 2020;20(8):1347-54. https://doi.org/10.2174/1871530320666200503052226

[27] Dahiya K, Aggarwal K, Seth S, et al. Type 2 diabetes mellitus without vascular complications and ischemia modified albumin. Clinical Laboratory Journal For Clinical Laboratories And Laboratories Related 2010;56(5):187.

[28] Ma S-G, Wei C-L, Hong B, et al. Ischemia-modified albumin in type 2 diabetic patients with and without peripheral arterial disease. Clinics 2011;66:1677-80.

[29] Kaefer M, Piva SJ, De Carvalho JAM, et al. Association between ischemia modified albumin, inflammation and hyperglycemia in type 2 diabetes mellitus. Clin Biochem 2010;43(4-5):450-4. https://doi.org/10.1016/j.clinbiochem.2009.11.018

[30] Piwowar A, Knapik-Kordecka M, Warwas M. Oxidative stress and endothelium dysfunction in diabetes mellitus type 2. Pol Merkur Lekarski. 2008;25(146):120-3.

[31] SavuO, Ionescu-TirgovisteC, AtanasiuV, GamanL, Papacocea R, Stoian I. Increase in total antioxidant capacity of plasma despite high levels of oxidative stress in uncomplicated type 2 diabetes mellitus. J Int Med Res 2012;40(2):709-16. https://doi.org/10.1177/147323001204000235

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ORIGINAL ARTICLE

The relationship between hypertension and COVID-19 vaccine in the long term and occupational evaluation

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ABSTRACT

Introduction: Many cardiovascular complications, especially myocarditis and pericarditis, have been observed with vaccination. One of these cardiovascular complications is hypertension with a rate of 1-5%. Hypertension emerged in the acute period after vaccination and tended to be persistent in patients with advanced age and comorbid diseases. In this study, we aimed to examine the relationship between hematological, biochemical markers and demographic characteristics between healthy individuals who have never been vaccinated or vaccinated and patients with new- onset hypertension.

Material and Method: Patients diagnosed with new-onset hypertension and healthy COVID-19 vaccinated, and non-COVID-19 vaccinated control patients of similar age and number were included in the study. The relationship between hematological, biochemical and demographic data between newly diagnosed hypertension patients and healthy COVID-19 vaccinated, and healthy non-vaccinated patients was examined.

Result: 56.3% of newly diagnosed hypertension patients were female, 46.9% were primary school graduates, 37.5% were housewives, 81.3% had stress in their lives. Hypertension patients were older ($p<0.01$), had lower hemoglobin levels ($p=0.05$) and higher LDL-C levels ($p<0.01$) than healthy unvaccinated patients.

Conclusion: Although it is seen that the cause of hypertension cannot be attributed solely to the vaccine, since the hypertensive group was older, had a higher body mass index, hyperglycemia and hyperlipidemia compared to the healthy group, and the number of patients in the study was small, but being a housewife is the most important occupational group and stress is an important trigger. The majority of vaccinees were primary school graduates. Anemia and LDL elevation were found in hypertensive and vaccinated patients. To the best of our knowledge, it is one of the first studies to examine LDL elevation in vaccinated patients and the relationship between long- term newly diagnosed hypertension and vaccination, although confounding factors cannot be excluded.

Key words: COVID-19, vaccine, hypertension, LD.

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INTRODUCTION

COVID-19 infection, a corona virus associated with SARS-CoV-2, which was first reported from China towards the end of 2019, was accepted as a pandemic by the World Health Organization (WHO) in March 2020 [1]. It affected 219 million people in the world and caused the death of 4.5 million people. Many vaccines such as inactive, mRNA, vector adeno virus have been produced to prevent it [2]. It is known that 68.5% of the world population has been vaccinated and 30% of them have received a reminder dose [3]. Many cardiovascular complications, especially myocarditis and pericarditis, have been observed with vaccination [2]. One of these cardiovascular complications is hypertension with a rate of 1-5% [4]. Hypertension emerged in acute periods after vaccination and tended to be permanent in patients with advanced age and comorbid diseases [5].

There are recent studies examining the relationship between vaccine and COVID-19. However, there is no study examining the relationship between long-term hypertension and vaccination. In
this study, we aimed to vaccination history in patients with new-onset hypertension, to examine other occupational and environmental exposures that may cause hypertension, and to examine the relationship between hematological, biochemical markers and demographic characteristics between healthy individuals who have never been vaccinated or vaccinated and patients with new-onset hypertension.

MATERIAL METHOD

Patients between the ages of 18 and 50, who applied to the internal medicine outpatient clinic of Mersin City Training and Research Hospital between November 01, 2023 and April 01, 2024 and whose blood pressure was found to be higher than 140/90 mm Hg in repeated measurements in the evaluations made by the internal medicine specialist and who were diagnosed with hypertension and who did not have additional diseases such as kidney disease, renovascular disease, hyperthyroid-hyperparathyroid- cushing etc. that may cause secondary hypertension. endocrinologic disease, obstructive sleep apnea syndrome [6] and control patients who applied to the Occupational and Occupational Diseases outpatient clinic on the same dates and who were evaluated by the Occupational and Occupational Diseases specialist with physical examination, hematologic, biochemical tests and imaging methods and no disease was detected were included in the study. Patients with hypertension for more than 6 months; patients with renal disease, renovascular disease, endocrinologic diseases such as hypertroid-hyperparathyroid-cushing's disease, obstructive sleep apnea syndrome, and [7] additional diseases that may cause hypertension secondary to hypertension will be excluded from the study. The relationship between hematologic, biochemical, demographic, occupational and vaccination status of newly diagnosed hypertension patients and healthy control patients will be examined.

Patient and healthy control groups; glucose, hematologic (hemoglobin [Hb], white blood cell [WBC], lymphocyte, neutrophil and biochemical parameters (creatinine, urea, aspartate transaminase [AST], alanine aminotransferase [ALT], triglyceride, high-density lipoproteins [HDL], low-density lipoprotein [LDL], fibrinogen, age, gender, height, weight, Body Mass Index [BMI], occupation, occupational risks (chemical, noise, pressure, cold, vibration), COVID-19 vaccination, how many doses, and which vaccines were obtained retrospectively by scanning the files.

The compatibility of the variables in the patient and control groups with normal distribution was examined using the Kolmogorov-Smirnov test. Mean ± SD (standard deviation) was used to define numerical variables and categorical variables were defined as number and percentage. The relationship between demographic, hematologic, biochemical values, occupation, occupational risks, vaccination status and doses between newly diagnosed hypertensive patients and healthy control group; t-test and Mann-Whitney U test were used to compare numerical variables, and Chi-square or Fisher's exact chi-square tests were used for categorical variables. Univariable and multivariable logistic regression was used to assess relationship between hypertension patients and other independent variables healthy vaccinated and unvaccinated patients. The significance level will be taken as $p \leq 0.05$.

Ethical approval was obtained from the non-interventional clinical ethics committee of Mersin University with the decision of the board dated 03 April 2024 and numbered 2024/343. The study was conducted in accordance with the Helsinki Principles. The

study was conducted in accordance with the Helsinki Principles.

RESULTS

Among the newly diagnosed hypertensive patients, 56.3% were female, mean age was 46.7±11.2 years, 84.4% had BMI >25, and 46.9% were obese. 46.9% were primary school graduates, 59.4% were non-smokers. 37.5% were housewives, 81.3% had frequent stress in their lives, 40.6% were exposed to cold and noise. Of the hypertension patients, 30 (93.8%) had COVID-19 vaccine, 11 (36.7%) had both vaccines, 19 (63.3%) had only BioNTech vaccine. There was no comorbid disease in the healthy vaccinated and unvaccinated control groups. In the hypertension group, 8 (25%) patients had additional diseases, 2 (6.25%) had diabetes mellitus, 2 (6.25%) had kidney disease, 2 (6.25%) had hypothyroidism and 2 (6.25%) had asthma, and no additional diseases such as malignancy were found. Since the study was a retrospective study, information about whether the patients had COVID-19, and their antibodies could not be obtained. All those with comorbid diseases were vaccinated and had hypertension. Demographic characteristics of hypertension patients is given Table 1. 20 (32.3%) of the vaccinated patients participating in the study received both vaccines, 1 (1.6%) only Sinovac, 41 (66.1%) only BioNTech.

Newly diagnosed hypertensive patients were overweight (p=0.03), had lower education levels (p=0.02), and higher urea (p=0.03) and creatinine (p=0.04) values than healthy and vaccinated patients. Newly diagnosed hypertension patients were older (p<0.01), overweight (p<0.01), had lower education levels (p<0.01), lower hemoglobin (p=0.05), glucose (p<0.01) and LDL-C (p<0.01) levels than healthy unvaccinated subjects.

Relationship between hypertension patients and healthy unvaccinated and healthy vaccinated patients is given Table 2.

Table 1. Demographic characteristics of hypertension patients

Features	N(%)
Age (years) mean±SD	46.7±11.2
Gender	
Female	18(56.3)
Male	14(43.6)
BMI kg/m^2 mean±SD	29.5 ± 6.1
18.5<	1(3.1)
18.5-25	4 (12.5)
25>	27(84.4)
30>	15(46.9)
Smoking	
Yes	13(40.6)
No	19(59.4)
Time Package/ Year Median (Min-Max)	0 (0-60)
Education	
Illiterate	2(6.3)
Primary School	15(46.9)
Secondary School	4(12.5)
High School	3(9.4)
University	8(25)
Systolic Blood Pressure mmHg mean±SD	153±16
Diastolic Blood Pressure mmHg mean±SD	93±10
Comorbidity	8(25)
Diabetes Mellitus	2(6.25)
Kidney Disease	2(6.25)
Hypothyroidism	2(6.25)
Asthma	2(6.25)
Occupation	
Housewife	12(37.5)
Officer	6(18.8)
Blue-Collar Worker	7(21.9)
Others	7(21.9)
Exposure	
Chemical	7(21.9)
Noise	13(40.6)
Vibration	13(40.6)
Stress	26(81.3)
Sleeplessness	10(31.3)
Cold	1(3.1)
Vaccination	30(93.8)
Single Dose	2(6.3)
2 Dose	8(25.0)
2> Dose	20(62.5)

BMI Body Mass Index

Table 2. Relationship between hypertension patients and healthy unvaccinated and healthy vaccinated patients

	Hypertansion n=32	Healthy Vaccinated n=32	P value	Healthy Unvaccinated n=32	P value
Features					
Age (years)	46.7±11.2	43.3±4.8	0.1	26.3±8.9	**<0.01**
Gender					
Female	18(56.3)	15(46.9)	0.5	10(31.2)	**0.04**
Male	14(43.8)	17(53.1)		22(68.8)	
BMI kg/m^2	29.6±6.1	26.6±3.8	**0.03**	24.5±5.5	**<0.01**
Education	2(6.3)	0(0)	**0.02**	0(0)	**<0.01**
Primary school	15(46.9)	10(31.3)		2(6.3)	
Secondary school	4(12.5)	2(6.3)		9(28.1)	
High school	3(9.4)	14(43.8)		13(40.6)	
University	8(25.0)	6(18.8)		8(25.0)	
Vaccination	30(93.8)	32(100)		0(0)	
Total dose median (min-max)	3(0-5)	3(1-5)	0.6	0(0-0)	**<0.01**
Sinovace dose median (min-max)	1(0-2)	1(0-3)	0.7	0(0-0)	**<0.01**
BioNTech dose median (min-max)	2(0-4)	2(0-4)	0.8	0(0-0)	**<0.01**
Hemoglobin g/dl mean±SD	13.9±1.9	14.1±1.5	0.5	14.8±1.9	**0.05**
Hematocrit % mean±SD	41.6±4.3	41.6±5.4	0.9	43.8±4.8	0.06
WBC x10^3 / uL mean±SD	8.0±2.2	8.0±2.0	0.9	7.5±1.7	0.3
Neutrophil x10^3 / uL mean±SD	4.6±1.7	4.9±1.9	0.6	4.5±1.4	0.8

BMI: Body Mass Index, HDL-C: High-Density Lipoprotein-Cholesterol, LDL-C: Low-Density Lipoprotein-Cholesterol

Table 3. The age- and gender-adjusted univariable and multivariable logistic regression analysis between hypertension patients and healthy unvaccinated patients.

Features	Univariable		Multivariable	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Age	0.8(0.7-0.9)	<0.01	0.8(0.7-0.9)	**0.02**
Gender	0.3(0.05-1.3)	0.1	0.2(0.02-2.9)	0.3
BMI	1.0(0.9-1.1)	0.9	0.9(0.8-1.2)	0.8
Hemoglobin	0.9(0.6-1.4)	0.9	0.9(0.4-1.7)	0.6
LDL-C	0.9(0.9-1)	0.04	1(0.9-1)	0.04

BMI: Body Mass Index, LDL-C: Low-Density Lipoprotein-Cholesterol

In the adjusted multivariable logistic regression analysis, older age (OR: 0.8, 95% CI: 0.7-0.9, p: 0.002), and higher LDL-C (OR: 1, 95% CI: 0.9-1, p: 0.004) were statistically significant in hypertension patients compared with healthy unvaccinated patients. The age- and gender-adjusted univariable and multivariable logistic regression analysis between hypertension patients and healthy unvaccinated patients is given Table 3.

DISCUSSION

The Centers for Disease Control and Prevention (CDC) reported that hypertension is more common in men with a rate of 50% worldwide [8], and the World Health Organization (WHO) emphasized that advanced age and obesity are risk factors for hypertension [9]. In studies conducted in our country, it was found that hypertension was more

common in women with a rate of 36.1% [10]. After the age of 40 years, there is an increase in susceptibility to atherosclerosis and the possibility of developing hypertension due to the decrease in vascular elasticity and the gradual decrease in estrogen with age, especially after the age of 45 years in women [11]. In our study, the rate was higher in women with a rate of 56.3%, the mean age was 46 years and 84.4% were overweight, which was similar to studies conducted in our country.

In our study, hypertension was found to be significantly higher in vaccinated patients than in healthy controls. However, since the hypertensive group was older, had a higher body mass index, hyperglycemia, and hyperlipidemia than the healthy group, the cause of hypertension cannot be attributed to the vaccine alone.

In studies conducted in developing countries, the rate of hypertension was found to be high in housewives, and it was emphasized that this was due to the fact that housewives have a stressful life due to taking care of the household, having a lot of work to do during the day and putting their own health and wishes in the second plan [11, 12]. Stress causes the development of hypertension over time by causing vasoconstrictor hormone release and even recurrent elevations in blood pressure [13], and work stress is also considered among the causes [14]. In addition, studies have shown that one of the most important risk factors for blood pressure is smoking and genetic burdens [15, 16] and in our study, 40.6% of hypertension patients were smokers. Although many factors such as smoking, genetic burden, and comorbid diseases cannot be excluded, housewifery was the most common occupational group, and stress was the most important risk factor.

In many studies including Turkey, a negative relationship was found between education and vaccination hesitancy [17-19]. However, in a large-scale study conducted in 15 different cities in Turkey using state data, no correlation was found between education level and vaccination, but a positive correlation was found between university and primary school graduates and a negative correlation was found between illiteracy and secondary school graduation [20]. In our study, 46.9% of those with hypertension who were vaccinated were primary school graduates and 40.6% of healthy unvaccinated individuals were high school graduates.

Many studies in the literature have shown that there is a positive correlation between high blood pressure and hemoglobin concentration [21,22]. In a study conducted on 4,203,887 people in Korea at the 3rd month after vaccination, the rate of nutritional anemia was found to be higher in those who were vaccinated and especially higher in those who received mRNA vaccination [23]. In our study, anemia was found in hypertension-vaccinated patients compared to unvaccinated healthy patients, and 63.3% of the vaccinated patients received biotech vaccine. Our study shows that although hemoglobin elevation was expected due to hypertension in patients, anemia was found in hypertensive and vaccinated patients, similar to the study conducted in Korea.

In a study conducted in Iran on lipid panel after vaccination, no effect of vaccination on lipid panel was found [24], and in a study conducted in Japan, elevated triglyceride levels were found [25]. In our study, LDL level was not found to be significant between hypertension-vaccinated patients and vaccinated-healthy control patients, whereas it was found to be significant in the unvaccinated-healthy control group; although confounding factors such as dietary behaviors and physical activity cannot be excluded, we found that vaccination has an elevating effect on LDL cholesterol. We think that these may be due to the disruption of the balance between LDL catabolism and production due to various reasons, such as proprotein convertase subtilisin or kexin type 9 (PCSK9), which has an important effect on hepatic receptor-mediated LDL catabolism, and angiopoietin-like protein 3 (ANGPTL3) [26], which regulates LDL production from its precursor very low-density lipoprotein.

The limitations of the study include the fact that it was a retrospective, single-center study; therefore, the number of patients was small and confounding factors such as dietary habits and physical activity, comorbid diseases, genetic factors, smoking could not be excluded.

Although it is seen that the cause of hypertension cannot be attributed solely to the vaccine, since the hypertensive group was older, had a higher body mass index, hyperglycemia and hyperlipidemia compared to the healthy group, and the number

of patients in the study was small, but housewifery was the most important occupational group and stress was an important trigger. The majority of those who received vaccination were primary school graduates. Anemia and elevated LDL levels were found in hypertensive and vaccinated patients. Although confounding factors cannot be excluded to the best of our knowledge, is one of the first studies to examine LDL elevation in vaccinated patients and the relationship between long-term newly diagnosed hypertension and vaccination.

Author contribution

Study conception and design: SME, TO; data collection: SME, TO; analysis and interpretation of results: SME; draft manuscript preparation: SME. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the non-interventional clinical ethics committee of Mersin University (Protocol no. 343/2024).

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] Lui DTW, Lee CH, Chow WS, et al. A territory-wide study on the impact of COVID- 19 on diabetes-related acute care [published online ahead of print, July 20, 2020]. J Diabetes Investig. 2020; https://doi.org/10.1111/jdi.13368.

[2] Ho JSY, Sia CH, Ngiam JN, Loh PH, Chew NWS, Kong W KF, et al.A review of COVID-19 vaccination and the reported cardiac manifestations. Singapore medical journal, 2023; 64(9):543-549.

[3] Buso G, Agabiti-Rosei,C, Muiesan M L. The relationship between COVID-19 vaccines and increased blood pressure: a word of caution. European Journal of Internal Medicine. 2023;111:27-29.

[4] Zappa M, Verdecchia P, Spanevello A, Visca D, Angeli F. Blood pressure increase after Pfizer/BioNTech SARS-CoV-2vaccine. Eur J Intern Med. 2021; 90 :111-113.

[5] Zhang V, Fisher M, Hou W, Zhang L, Duong TQ. Incidence of new-onset hypertension post-COVID-19: comparison with influenza. Hypertension. 2023; 80(10), 2135-2148.

[6] [Endocrinology and Metabolism Society of Turkey. Hypertension Diagnosis and Treatment Guideline]. Ankara. 2022. Access Date: 26 May 2024. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://file.temd.org.tr/Uploads/publications/guides/documents/Hipertansiyon-Kilavuzu-2022.pdf

[7] Puar THK, Mok Y, Debajyoti R, Khoo J, How CH, et al. Secondary hypertension in adults. Singapore medical journal. 2016; 57(5): 228.

[8] Centers for Disease Control and Prevention. High Blood Pressure. July 6, 2023. Available: 23 March 2024. https://www.cdc.gov/bloodpressure/facts.htm

[9] World Health Organization. Hypertension. 16 March, 2023. Available: 23 March 2024. https://www.who.int/news-room/fact-sheets/detail/hypertension.

[10] Bayram F, Demir Ö, Sabuncu T, Eren MA, Gedik AV, Çorapçıoğlu D, et al. Prevalence and Awareness of Hypertension in Seven Distinct Geographic Regions of Turkey: The SEMT HT Study. Turk J Endocrinol Metab. 2021; 25:1-10.

[11] Andria KM, Widati S, Nurmala I. The Characteristics of Hypertension Patients at Puskesmas Waru, Pamekasan in 2018. Jurnal Promkes. 2021; 9(1): 11.

[12] De S, Vidyapeeth B, Ray SP, Mahangare MK, Jadhav MP, Thorat M P, Malkapurkar MS. Knowledge and Risk Factors Of Hypertension Among Housewives. The Journal Of Oriental Research Madras. 2021; XCII(XLII1),28-142

[13] Kulkarni S, O'Farrell I, Erasi M, Kochar MS. Stress and hypertension. WMJ: official publication of the State Medical Society of Wisconsi. 1998; 97(11), 34-38.

[14] Rosenthal T, Alter A. Occupational stress and hypertension. Journal of the American Society of Hypertension. 2012; 6(1): 2-22.

[15] Virdis A, Giannarelli C, Fritsch NM, Taddei S , Ghiadoni L. Cigarette smoking and hypertension. Current pharmaceutical design. 2010; 16(23): 2518-2525.

[16] Padmanabhan S, Caulfield M, Dominiczak AF. Genetic and molecular aspects of hypertension. Circulation research. 2015;116(6): 937-959.

[17] Schwarzinger M, Watson V, Arwidson P, Alla, F, Luchini L. COVID-19 Vaccine Hesitancy in a Representative Working-Age Population in France: A Survey Experiment Based on Vaccine Characteristics. Lancet Public Health. 2021; 6: e210- e221.

[18] Nguyen KH, Nguyen K, Corlin L, Allen JD, Chung M. Changes in COVID-19 Vaccination Receipt and Intention to Vaccinate by Socioeconomic Characteristics and Geographic Area, United States, January 6-March 29, 2021. Ann. Med. 2021; 53:1419-1428.

[19] Sonmezer MC, Sahin TK, Erul E, Ceylan FS, Hamurcu MY, Morova N, et al. Knowledge, Attitudes, and Perception towards COVID-19 Vaccination among the Adult Population: A Cross-Sectional Study in Turkey. Vaccines 2022; 10:278.

[20] Cengiz B, Sayılır MÜ, Zengin NY, Küçük ÖN, Soylu AR. Does the COVID-19 vaccination rate change according to the education and income: a study on vaccination rates in cities of Turkey between 2021-September and 2022-February. Vaccines. 2022; 10(11): 1933.

[21] Kawamoto R, Tabara Y, Kohara K, et al. A slightly low haemoglobin level is beneficially associated with arterial stiffness in japanese community-dwelling women. Clinical and Experimental Hypertension. 2012;34(2):92-98. https://doi.org/10.3109/10641963.2011.618202.

[22] Atsma F, Veldhuizen I, de Kort W, van Kraaij M, Pasker-de Jong P, Deinum JJH. Haemoglobin level is positively associated with blood pressure in a large cohort of healthy individuals. Hypertension. 2012;60(4):936-941. https://doi.org/10.1161/hypertensionaha.112.193565

[23] Choi HS, Kim MH, Choi MG, Park JH, Chun EM. Haematologic abnormalities after COVID-19 vaccination: A large Korean population-based cohort study. MedRxiv. 2023; 2023:11.

[24] Al-Salmi TGR, Al-Maamori JAI. The Biochemical Assessments of Cellular Oxidant- Antioxidant Status and Lipid Profile for Some of COVID-19 Vaccines (Pfizer, AstraZeneca and Sinopharm) in Vaccinated People of Wasit Province in Iraq. Journal of Pharmaceutical Negative Results. 2022:70-78.

[25] Islam Z, Yamamoto S, Mizoue T, Oshiro Y, Inamura N, Konishi M, et al. Dyslipidaemia and SARS-CoV-2 spike antibody titres after the second and third doses of the BNT162b2 vaccine among healthcare workers in Japan. Diabetes/Metabolism Research and Reviews. 2023; 39(3): e3606.

[26] Adam RC, Mintah IJ, Alexa-Braun CA, et al. Angiopoietin-like protein 3 governs LDL-cholesterol levels through endothelial lipase-dependent VLDL clearance. J Lipid Res 2020; 61: 1271-86.

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ORIGINAL ARTICLE

A tale of two uropathologists: concordance of Gleason Grade Groups in prostatic adenocarcinoma over needle biopsies and radical prostatectomies

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ABSTRACT

Objective: The standard 12-core transrectal prostate needle biopsies don't reflect the tumor entirely. Approximately 35-36% of needle biopsy diagnoses of Gleason grade group (GG) 1 are upgraded upon radical prostatectomy (RP). Pathologists are not in perfect concordance in Gleason scoring. Two uropathologists in a university hospital aimed to determine how concordant needle biopsy GGs were with RP GGs. Moreover, they also assessed how frequently they up-/down-graded the needle biopsy GGs each other gave when they graded RPs.

Material and Methods: In-house prostate needle biopsies and RPs from 31/01/2020 to 10/09/2022 were retrieved from the hospital database. Patients who had both a needle biopsy and an RP were included. Whether each case was down-/up-graded upon RP, GGs and the pathologist that reported the cases were tabulated.

Results: One hundred and thirty cases were assessed. Needle biopsy and RP GGs were identical in 63,1% (n=82). Pathologist1 (P1) assessed both the needle biopsy and RPs of 41 patients, 8 of which they downgraded and 8 upgraded (19,5%). Pathologist2 (P2) assessed both the needle biopsy and RP samples of 23 patients; downgrading 13% (n=3) and upgrading 17,4% (n=4) of cases. Where the needle biopsy was reported by P2 and RP was reported by P1 (n=48), 10 (20,8%) were downgraded and 8 (16,7%) were upgraded. The reverse scenario was noted in 18 patients; 2 (11,1%) of which were downgraded, 5 (27,8%) were upgraded. While P2 showed a tendency to upgrade more frequently, this was not statistically significant (p=0,2774, Pearson chi-square).

Discussion: The two uropathologists' up- and down-grading rates seemed concordant. Routine practice doesn't allow time for one pathologist to re-score the other's cases, nor is every case consulted. Looking back at pathologist-specific tendencies to up/downgrade one's own or a colleague's scores may help direct ourselves and others to curb tendencies to over/undergrade.

Keywords: prostatic adenocarcinoma, gleason grade group, concordance, upgrading, downgrading, needle biopsy, radical prostatectomy

INTRODUCTION

Accurate grading of prostatic adenocarcinoma is of paramount importance for treatment decisions and prognostication. Gleason grading is decisive for treatment in about 40% of all prostate cancer patients [1]. Laboratory grading practices may impact treatment decisions [2].

Needle biopsy Gleason scores tend to differ from that of the radical prostatectomy Gleason scores in a considerable portion of cases. The routine 12-core biopsy scheme showed a biopsy-radical Gleason score concordance rate as high as 85% with a possibility of upgrading at 17% of cases and downgrading of 14% [3]. UK data indicate rates of 25.5% and 15.6% for upgrade and downgrading, respectively; their concordance rate is 58.9% [4]. In another study, about one third of Gleason score 6 cases were upgraded at radical prostatectomy; about one third of Gleason score 8 cases got upgraded and another third got downgraded [5]. More than a third (38.2%) of cases with Gleason score 6 at biopsy got upgraded at radical prostatectomy [6]. Several factors affect down- and up-grading tendencies such as needle biopsy procedures themselves [7], cancer volume/extent at biopsy [8, 9], PSA levels [5, 8, 9], prostate volume [5, 8], the presence of extraprostatic extension and surgical margin positivity [9], risk group of disease [4, 10], and the presence of a tertiary Gleason pattern [5]. Lower D'Amico risk groups tend to have a higher rate of upgrading [4]; however, the opposite is also claimed [10]. Magnetic resonance imaging (MRI) - guided biopsies are more sensitive and less specific than transrectal US-guided biopsies to detect clinically relevant prostate cancer [7], MRI-targeted biopsies and systematic (12-core) biopsies have a similar downgrading risk while MRI-targeted sampling has a lower risk of upgrading [11]. Properties of the population under study (low vs high clinical risk / mixed), Gleason scoring systems used (modified vs previous), tumor and organ properties (high vs low extent tumor, high vs low prostate volume), sampling types (targeted vs systematic needle sampling, transrectal vs transperineal [12]) all vary among studies; yet overall it is safe to comment that needle biopsy Gleason scores tend to differ from that of the radical prostatectomy Gleason score in about one fourth to one third of all cases. This has prognostic and therapeutic implications; upgrading on radical prostatectomy is associated with increased risk of biochemical recurrence and adverse pathological parameters [10]. Undergraded cases may be undertreated, overgraded cases can be overtreated [4].

Gleason grading scheme is arguably the best described grading system in all surgical pathology; yet interobserver variability remains. In the early 2000s, North American general pathologists showed barely moderate agreement (kappa=0,435) while uropathologists were in moderate to substantial agreement (kappa=0,56-0,70) [13]. British pathologists in 2006 followed with moderate overall interobserver agreement (kappa=0,54); their intra-observer agreement was good (kappa=0,66) [14]. Turkish general pathologists showed barely moderate agreement both before and after the Gleason grade modification [15] (kappa=0,45 [16], kappa=0,43 [17]). Others report similar extents of interobserver agreement (Kappa=0,482 [18], kappa=0,55 [19]) with outliers (kappa= 0,753 [20]). Poorly differentiated/high grade tumors may have better overall agreement (kappa=0,65) than well differentiated/low grade tumors (kappa=0,15) [21]. Low tumor volumes in needle biopsies [22, 23] and distinguishing Gleason score 6 and 7 with low pattern 4 percents tend to be the most challenging aspects [23].

Interobserver agreement studies typically include re-assessment of previously reported samples; this is not easily achievable and can't be repeated frequently in busy pathology practices. Needle biopsy - radical prostatectomy Gleason score variation per patient is also a variable in routine pathology work that needs consideration. The present study aims to combine these two sources of inconsistency/variability in an easily evaluated manner and assess 1) the needle-to-radical Gleason scoring changes of two uropathologists in the same academic institution in Turkey, and 2) attempt to evaluate how concordant these two pathologists are without reassessment of previous samples/ slides and complicated statistical calculations.

METHODS

All in-house prostate needle biopsies and in-house radical prostatectomies between 31st January 2020 and 10th September 2022 of one university hospital were listed using the digital hospital database. Patients who underwent both a needle biopsy and an RP at our institution were included in the study. The highest GG detected in the needle biopsy and the GG of the dominant nodule in RP were noted for each patient. Minor/tertiary Gleason patterns in RPs were not evaluated. Whether each case was down- or upgraded upon radical prostatectomy and the pathologist that signed out the cases were assessed. The percentages and numbers of cases each pathologist up- or downgraded were calculated. Cases where one pathologist signed out the needle biopsy and the other reported the radical prostatectomy were separately assessed to see whether there was a tendency of up or downgrading in any of the two scenarios. Numbers of cases were tabulated whenever possible, and Pearson chi-square test was applied to detect statistically significant associations for 2x2 tables (http://vassarstats.net/, accessed 08/29/23). Fisher's Exact Test was used for larger tables (https://astatsa.com/FisherTest/, accessed 07/31/2024). A p value <0.05 was considered significant.

No patient identifier was included in the data; the pathology reports along with any associated human tissue/blocks remained unchanged and intact. The study was approved by the Health Sciences Research Ethics Committee of the Hacettepe University Hospital (Protocol no. SBA 23/239).

RESULTS

One hundred and thirty patients had both their needle biopsies and prostatectomies reported in our department between 31st January 2020 and 10th September 2022. The distribution of cases between Pathologist 1 (P1) and Pathologist 2 (P2) are given in Table 1. The breakdown of discordant (up- or downgraded) cases are given in Table 2.

Overall concordance between needle biopsy and radical prostatectomy GGs was 63,1%. P2 assessed both the needle biopsy and radical prostatectomy samples of 23 patients; downgraded three cases (13%) and upgraded 4 (17,4%). P1 assessed both the needle biopsy and radical prostatectomy samples of 41 patients, downgraded and upgraded 19,5% each. As is seen in Table 1, P1 up/downgraded their own needle biopsy GGs in 16 cases (39%); P2 up/downgraded their own needle biopsy GGs in 7 cases (30,4%). Of the 59 patients whose needle biopsies were reported by P1, 10 (16,9%) were downgraded and 13 (22%) were upgraded upon radical prostatectomy. Seventy-one needle biopsies were reported by P2; 13 (18,3%) were downgraded and 12 (16,9%) were upgraded upon radical prostatectomy. Overall, the possibility of P1's needle biopsy GG getting changed at radical prostatectomy was 38,98% (23/59) and for P2 the same possibility was 35,21% (25/71).

Where the needle biopsy was reported by P2 and the radical prostatectomy was reported by P1 (n=48), 10 (20,8%) were downgraded and 8 (16,7%)

Table 1. Distribution of cases

Needle biopsy reported by	Radical prostatectomy reported by	Number of cases downgraded (n, %)	Number of cases upgraded (n, %)	Number of cases with no change in GG (n, %)	Total
P1	P1	8 (19,5%)	8 (19,5%)	25 (61%)	41 (100%)
P1	P2	2 (11,1%)	5 (27,8%)	11 (61,1%)	18 (100%)
P2	P1	10 (20,8%)	8 (16,7%)	30 (62,5%)	48 (100%)
P2	P2	3 (13%)	4 (17,4%)	16 (69,6%)	23 (100%)
	Total	23 (17,7%)	25 (19,2%)	82 (63,1%)	130

p= 0.9251 Fisher's Exact Test, 2-sided

Table 2. The tendency of each pathologist to down/upgrade a needle biopsy grade upon radical prostatectomy

Cases down/upgraded by	Downgraded (n, %)	Upgraded (n, %)	Total
P1	18 (78,3%)	16 (64%)	34
P2	5 (21,7%)	9 (36%)	14
Total	23 (100%)	25 (100%)	48

p=0.2774 Pearson chi square

were upgraded. The reverse scenario was noted in 18 patients; 2 (11,1%) of which were downgraded, 5 (27,8%) were upgraded. While P2 showed a tendency to upgrade more frequently, this was not statistically significant (p=0.2774, Pearson chi-square).

DISCUSSION

Gleason grading system is one of the most important prognostic factors for prostatic adenocarcinomas. It is known that about one third or one fourth of cases undergo a Gleason grading change between their needle biopsies and radical prostatectomies; noting a sampling issue in Gleason grading. Another source of variation is interobserver variability, in which most reports indicate moderate concordance between pathologists. These two major sources of variability in prostate carcinoma Gleason grading (interobserver and sampling variabilities) are evaluated and reported separately using labor-intensive studies. The present study aimed to demonstrate the cumulative effect of these two factors in the routine workflow of one uropathology practice of two pathologists without microscopic re-reviewing of past slides or statistical calculations of kappa values.

The total number of cases whose needle biopsy samples and radical prostatectomies were assessed at the same institution (university hospital) in a span of 2 years was 130. About two thirds of these cases have not undergone up- or downgrading (82; 63,1%); which seems to be concordant with relevant Turkish literature [24, 25]. As the up- and downgrading rates are very much alike (25 - 19,2% and 23 - 17,7%, respectively); institution-wise, the present set of pathologists do not seem to be over-upgrading or over-downgrading. Some laboratories may tend to grade lower or higher than average [2]. The up and downgrading rates calculated in the present study are similar to those reported by Bjurlin et al (17% up and 14% downgrading [3]); and lower than that of most of the pertinent literature [4-6].

P2 tended to have lower rates of downgrading than P1 (5 vs 18 cases, Table 2), yet this tendency did not reach statistical significance. This may mean that individual pathologist variability may not substantially impact overall grading consistency. Evaluating a larger patient group and a longer period might help support or refute this tendency.

The present study has several drawbacks beyond its retrospective design. Relatively low numbers of patients in the present study precludes detailed analyses of other parameters claimed to affect rates of upgrading such as PSA levels and prostate volume [26] along with Gleason grade groups themselves. Prognostic data, such as biochemical recurrence, metastasis, dead of disease, etc. was not assessed. Discordant (up/downgraded) needle biopsy - radical prostatectomy pairs were not re-assessed by both pathologists to turn this endeavor to an educational opportunity for minimizing interobserver variability. However, the tables in the results section can be adapted to any lab and reproduced by almost any healthcare worker (nurse, medical secretary, medical student, etc), creating an ongoing and temporally evolving database of up and downgrading tendencies that can eventually be used for discussions and create teaching opportunities. Future studies that have larger sample sizes and include prognostic outcomes may help combine the two factors of inconsistency in Gleason grading (interobserver variability and variations inherent to sampling) to validate or refute these findings.

Author contribution

Study conception and design: GG, KK; data collection: GG; analysis and interpretation of results: GG, KK; draft manuscript preparation: GG, KK. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Health Sciences Research Ethics Committee of the Hacettepe University Hospital (Protocol no. SBA 23/239).

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Prostate Cancer Version 3.2023 National Comprehensive Cancer Network®; 2023 [updated 08/07/2023. Version 3.2023. Available from: chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.nccn.org/professionals/physician_gls/pdf/prostate.pdf.

[2] Flach RN, van Dooijeweert C, Aben KKH, et al. Interlaboratory Gleason grading variation affects treatment: a Dutch historic cohort study in 30 509 patients with prostate cancer. J Clin Pathol. 2023; 76(10):690-697.

[3] Bjurlin MA, Carter HB, Schellhammer P, et al. Optimization of initial prostate biopsy in clinical practice: sampling, labeling and specimen processing. J Urol. 2013;189(6):2039-46.

[4] Bullock N, Simpkin A, Fowler S, et al. Pathological upgrading in prostate cancer treated with surgery in the United Kingdom: trends and risk factors from the British Association of Urological Surgeons Radical Prostatectomy Registry. BMC Urol. 2019;19(1):94.

[5] Epstein JI, Feng Z, Trock BJ, et al. Upgrading and downgrading of prostate cancer from biopsy to radical prostatectomy: incidence and predictive factors using the modified Gleason grading system and factoring in tertiary grades. Eur Urol. 2012;61(5):1019-24.

[6] Milonas D, Grybas A, Auskalnis S, et al. Factors predicting Gleason score 6 upgrading after radical prostatectomy. Cent European J Urol. 2011;64(4):205-8.

[7] Ahmed HU, El-Shater Bosaily A, Brown LC, et al. Diagnostic accuracy of multi-parametric MRI and TRUS biopsy in prostate cancer (PROMIS): a paired validating confirmatory study. Lancet. 2017;389(10071):815-22.

[8] Dong F, Jones JS, Stephenson AJ, et al. Prostate cancer volume at biopsy predicts clinically significant upgrading. J Urol. 2008;179(3):896-900.

[9] Hong SK, Han BK, Lee ST, et al. Prediction of Gleason score upgrading in low-risk prostate cancers diagnosed via multi (> or = 12)-core prostate biopsy. World J Urol. 2009;27(2):271-6.

[10] Freedland SJ, Kane CJ, Amling CL, et al. Upgrading and downgrading of prostate needle biopsy specimens: risk factors and clinical implications. Urology. 2007;69(3):495-9.

[11] Goel S, Shoag JE, Gross MD, et al. Concordance Between Biopsy and Radical Prostatectomy Pathology in the Era of Targeted Biopsy: A Systematic Review and Meta-analysis. Eur Urol Oncol. 2020;3(1):10-20.

[12] Marra G, Eldred-Evans D, Challacombe B, et al. Pathological Concordance between Prostate Biopsies and Radical Prostatectomy Using Transperineal Sector Mapping Biopsies: Validation and Comparison with Transrectal Biopsies. Urol Int. 2017;99(2):168-76.

[13] Allsbrook WC, Jr., Mangold KA, Johnson MH, et al. Interobserver reproducibility of Gleason grading of prostatic carcinoma: general pathologist. Hum Pathol. 2001;32(1):81-8.

[14] Melia J, Moseley R, Ball RY, et al. A UK-based investigation of inter- and intra-observer reproducibility of Gleason grading of prostatic biopsies. Histopathology. 2006;48(6):644-54.

[15] Epstein JI, Zelefsky MJ, Sjoberg DD, et al. A Contemporary Prostate Cancer Grading System: A Validated Alternative to the Gleason Score. Eur Urol. 2016;69(3):428-35.

[16] Dere Y, Celik OI, Celik SY, et al. A grading dilemma; Gleason scoring system: Are we sufficiently compatible? A multi center study. Indian J Pathol Microbiol. 2020;63(Supplement):S25-S9.

[17] Ozkan TA, Eruyar AT, Cebeci OO, et al. Interobserver variability in Gleason histological grading of prostate cancer. Scand J Urol. 2016;50(6):420-4.

[18] Qureshi A, Lakhtakia R, Al Bahri M, et al. Gleason's Grading of Prostatic Adenocarcinoma: Inter-Observer Variation Among Seven Pathologists at a Tertiary Care Center in Oman. Asian Pac J Cancer Prev. 2016;17(11):4867-8.

[19] Singh RV, Agashe SR, Gosavi AV, et al. Interobserver reproducibility of Gleason grading of prostatic adenocarcinoma among general pathologists. Indian J Cancer. 2011;48(4):488-95.

[20] Al Nemer AM, Elsharkawy T, Elshawarby M, et al. The updated grading system of prostate carcinoma: an inter-observer agreement study among general pathologists in an academic practice. APMIS. 2017;125(11):957-61.

[21] Bori R, Salamon F, Moczar C, et al. Interobserver reproducibility of Gleason grading in prostate biopsy samples. Orv Hetil. 2013;154(31):1219-25.

[22] Sadimin ET, Khani F, Diolombi M, et al. Interobserver Reproducibility of Percent Gleason Pattern 4 in Prostatic Adenocarcinoma on Prostate Biopsies. Am J Surg Pathol. 2016;40(12):1686-92.

[23] Coard KC, Freeman VL. Gleason grading of prostate cancer: level of concordance between pathologists at the University Hospital of the West Indies. Am J Clin Pathol. 2004;122(3):373-6.

[24] Akarken İ, Dere Y, Tarhan H, et al. Assessment of gleason score concordance between prostate biopsy and radical prostatectomy. J Cukurova Anesth Surg. 2022;5(2):274-9.

[25] Öztürk EY, Yıkılmaz TN. Gleason score correlation between prostate biopsy and radical prostatectomy specimens. Bulletin of Urooncology. 2018;17(1):1-4.

[26] Tilki D, Schlenker B, John M, et al. Clinical and pathologic predictors of Gleason sum upgrading in patients after radical prostatectomy: results from a single institution series. Urol Oncol. 2011;29(5):508-14.

acta medica

ORIGINAL ARTICLE

The assesment of dermatology life quality index in nurses with occupational skin diseases in Türkiye

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ABSTRACT

Objective: The skin acts as one of the body's first defense components against many external factors by creating a physical barrier. Therefore, it is significantly vulnerable to the irritating effects of protective personal equipment use. A wide range of occupational skin diseases (OSD) can be observed in the nurses who are in close contact with patients and frequently use personal equipment. We aim to identify different types OSD observed in the nurses and explore the impact of these skin diseases upon Dermatology Life Quality Index (DLQI).

Materials and Methods: A web-based questionnaire consisting of 24 questions related to the physician-confirmed cutaneous diseases observed in the participants who were actively working as nurses in Türkiye. DLQI was calculated by using DLQI score.

Results: Two hundred twenty nine participants were included in the study with a mean age of 33.74 years. The mean duration working as a nurse was 9.86 years. One hundred (43.7%) participants reported to have at least one skin disease; the most commonly observed occupational cutaneous skin diseases were xerosis, contact dermatitis, pruritus, brittle nail syndrome and callus formation. The median DLQI score was 4 (interquartile range=7.5). The time during which the participants worked as a nurse was significantly associated with the development of at least one OSD (p=0.02).

Conclusions: Our study show that OSD, most common ones being xerosis, contact dermatitis and pruritus seem to affect dermatologic life quality of the nurses in Türkiye.

Keywords: nursing, skin, surveys and questionnaires.

INTRODUCTION

Nurses are occupationally exposed to a wide variety of allergens such as protective gloves, disinfectants and medications. This condition, called occupational contact dermatitis, has a prevalence of up to 30%, especially among nurses and other healthcare professionals [1]. The most common occupational skin diseases (OSD) observed among the healthcare workers are reported to be chronic irritant contact dermatitis (ICD) and allergic contact dermatitis (ACD) [2].

Especially during the COVID-19 pandemic, with the increase of the frequency and duration of the use of protective personal equipment (PPE), many new-onset skin diseases have been observed in healthcare workers [3]. Daye et al. [3] reported that the most common skin diseases observed in healthcare workers during the pandemic period were dryness, itching, burning, peeling and lichenification, and 22.3% of the participants stated that the use of PPE increased the severity of their

pre-existing skin diseases. In this study, it was also reported that the frequency of acne has increased due to long-term use of protective masks, and the Dermatology Life Quality Index (DLQI) was reported to be significantly elevated, especially in women [3]. This study shows that there is an increased risk of developing OSD in the healthcare personnel, especially during the periods of more intense work such as pandemics during which the duration of PPE use is significantly extended.

In our study, we aimed to determine the types and frequencies of OSD observed in the nurses working in different institutions and various units affiliated with these institutions in our country. We also planned to disclose the effect of the OSD observed in nurses on the DLQI.

MATERIAL and METHODS

Design

This cross-sectional investigation study included subjects of >18 years who were currently working as a nurse at the time of the study. The study questionnaire was spread among the individuals who were employed in the nursing sector in different institutions in Türkiye between June and July 2024.

Ethical Approval

Before the start of the present study, approval of local non-interventional studies ethics committee was obtained (the date and decision number: May 9 2024, 2024/018). This study protocol was consistent with the Declaration of Helsinki and all participants gave informed consent to participate into the study.

A web-based questionnaire which composed of 24 questions was formed by Google forms (Google LLC, USA). This questionnaire was spread out via instant messaging or e-mail and the virtual snowball sampling method was used to convey the study. The study was filled out by the nurses who were working in the different units of various health care centers in Türkiye. The evaluation details were grouped into four sections in the survey: (I) demographical and work-related data of the nurses (gender, age,time spent as a nurse, chronic diseases current institution/unit, the presence of any physician-confirmed OSD) (II) the type of OSD (xerosis, hand dermatitis, miliaria, hyperpigmentation on the hands, paronychia, orolabial herpes, facial acne due to mask use, folliculitis, skin burn due to occupational exposure, perioral dermatitis, brittle nail syndrome, onychoschizia/onychorrhexis, onycholysis, skin atrophy, pruritus, palmar or plantar warts, palmoplantar peeling, airborne contact dermatitis, others) (III) DLQI scoring4 questions (10 item) (IV) treatment status for dermatologic disease.

Statistical Analysis

IBM SPSS 29.0 program was used for the analysis of the data. Qualitative variables are shown as frequencies and percentages; whereas quantitative variables are shown with mean, standard deviation (SD), median, interquartile range (IQR), quartile1-3 values.

Spearman's correlation testwas used to explore the direct association between two numerical variables with non-normal distribution whereas the association between categorical variables were determined via Chi-square test.

Binary logistic regression analysis was used to investigate the relationship between the target dependent variable and one or more independent variables. $p<0.05$ was accepted to be statistically significant.

RESULTS

The study included 229 participants with a mean age of 33.74 years (SD)=8.62 and female predominance (86%). The average duration during which the participants have worked as a nurse was 9.86 years (SD=9.34).

One-hundred three (44.9%) were working in government hospitals, 38 (16.6%) in university hospitals, 33 (14.4%) in research and training hospitals, 21 (9.2%) in family health centers whereas 6 (2.6%) and 19 (8.3%) were working in private hospitals and other institutions (community health centers, schools etc.), respectively. If we were to look at the individual institution units, 51(22.4%) subjects were responsible for inpatient service, 44 (19.3%) for polyclinic, 27 (11.8%) for emergency department, 23 (10.1%) for operating room, 18

(7.9%) for vaccination, 12 (5.3%) for intensive care unit (ICU), 12 (5.3%) for community health-related department and 42 (18.5%) in other units (delivery room, infirmary, etc.).

Only 100 (43.7%) subjects were reported to have at least one OSD which was confirmed by a physician. The most commonly reported ones were xerosis (74.8%) followed by pruritus (49.6%) and hand dermatitis (43.5%). The distribution of the OSD is shown in Figure 1. Seven (7%) subjects presented with at least one skin disease were also diagnosed with hypothyroidism, six (6%) ones had a diagnosis of allergic asthma whereas two (2%) had allergic rhinitis and one (1%) had chronic urticaria. Only fourty two (23.1%) participants were currently under treatment for their dermatological disease.

DLQI was calculated for the participants who reported to be affected by at least one cutaneous disease, by using DLQI questionnaire.The mean DLQI score was 5.96 (SD=6.32) whereas median DLQI score was found to be 4 and IQR was 7.5 (Figure 2). Twenty four (24%) participants had a DLQI score of 0-1 (no effect on the person's life) and 76 ones had a score of ≥ 2.

No significant relationship was found between the type units that the participants have been working and the presence of any OSD (Chi-square test, p=0.14).

There was no statistically significant relationship between the number of years working as a nurse and DLQI scoring [Spearman's coefficient= 0.14 (low correlation) and p=0.17] (Figure 3).

Binary logistic regression analysis showed that gender, age,workplace,were not associated with a significant risk of developing a skin disease (odds ratio:0.98; 95% confidence interval: 0.45-2.11; p=0.95 for gender, odds ratio: 0.98; 95% confidence interval: 0.94-1; p=0.06 for age,odds ratio: 0.98; 95% confidence interval: 0.86-1.11; p=0.78 for workplace. When the participants were divided into two groups as ICU nurses and non-ICU nurses, logistic regression analysis still didn't show any significant correlation between being ICU nurse and having an OSD (odds ratio: 0.34; 95% confidence interval:0.12-1.03; p=0.06). However, the years worked as a nurse seemed to be significantly associated with developing an OSD (odds ratio:1.04; 95% confidence interval: 1.01-1.07; p=0.02).

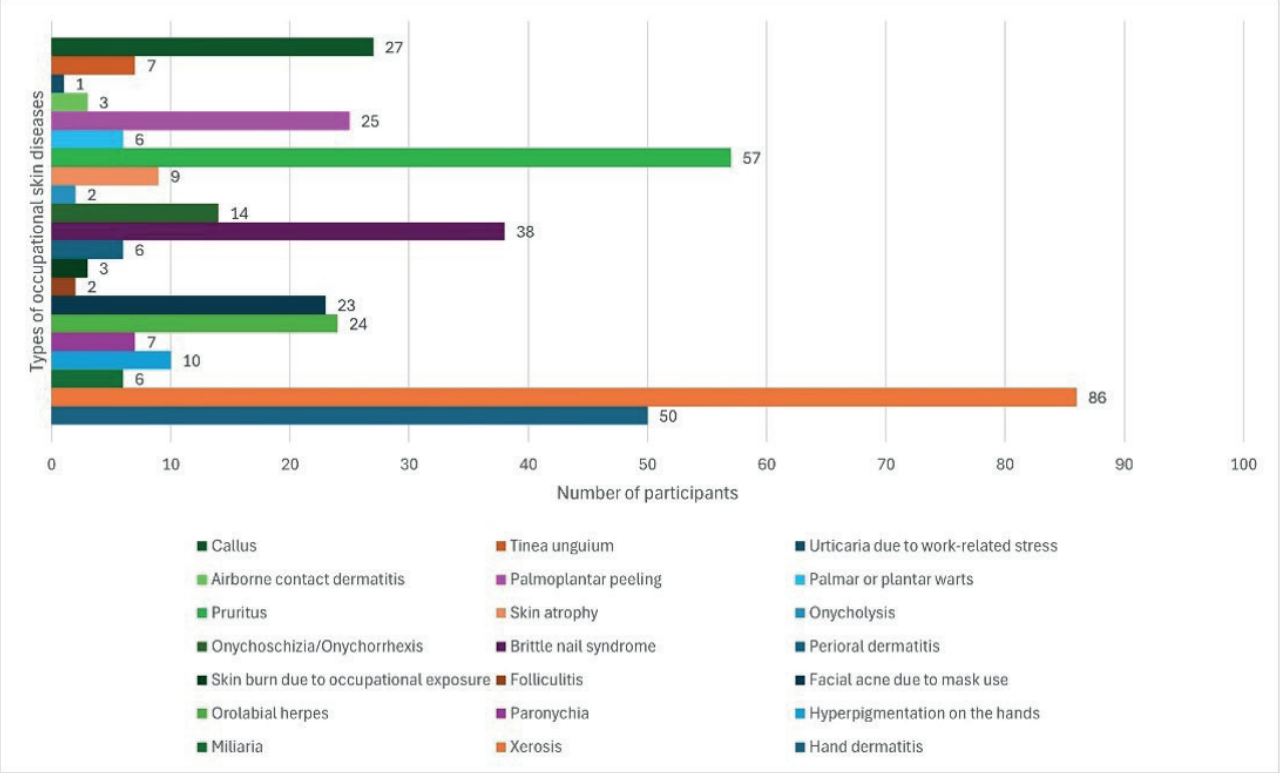

Figure 1. The distribution of cutaneous skin diseases seen in the participants

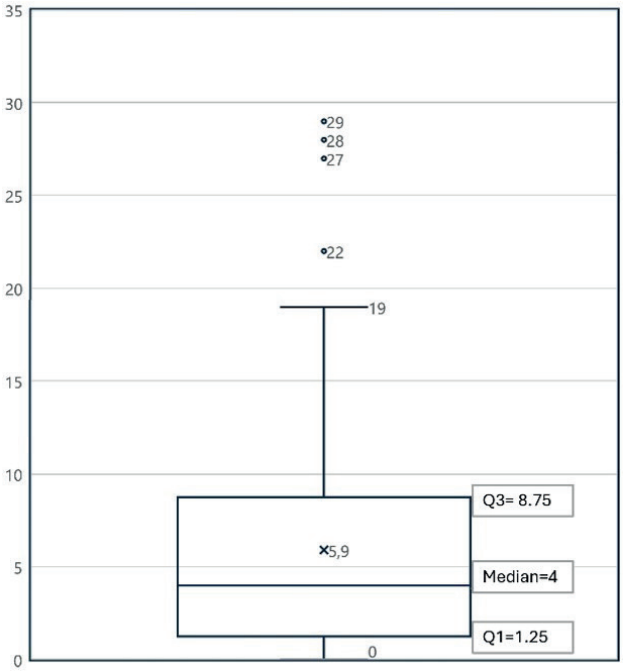

Figure 2. Boxplot representing median values, 25–75% range (box) and minimum–maximum range (bars) of DLQI score

DISCUSSION

In the present study, 43.7% of the participants reported to have at least one OSD; the most common ones being xerosis, hand dermatitis, pruritus, brittle nail syndrome and callus. The median DLQI was found to be 4 which falls into the category of 'small effect on patient's life'. The results show that nearly half of the nurses participated into our study are influenced by at least one OSD. Even though no statistically significant association was detected between the number of years working as a nurse and DLQI score; the duration during which the participants worked as a nurse seemed to be significantly correlated with developing at least one OSD.

Work-related skin diseases seem to be a common, emerging problem among healthcare workers.ACD andICD are the most prevalent OSD [5]. Nurses who are exposed to various allergens such as rubber glove chemicals and hand cleansers due to intense, prolonged working hours, are prone to develop numerous OSD [2]. In a study by Kieć-Swierczyńska and Krecisz [6], the prevalence of contact allergy was reported to be 66.4%, whereas another research from a USA hospital showed that55% of

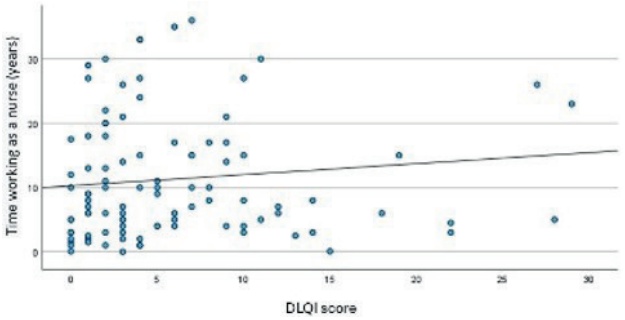

Figure 3. Scatter plotof nursing time (years) vs. DLQI score

the nurses had symptoms of hand dermatitis with a prevalence of 65% among the nurses who worked in ICU and a prevalence of 50% among non-ICU nurses [7]. Rubber glove chemicals, antiseptics and preservatives are the most commonly blamed substances in the etiopathogenesis of ACD in health care workers [2]. On the other hand, occupational ICD is linked to the irritating effects of wet, sweat, heat and hand sanitizers [2]. In our study, the prevalence of hand dermatitis was 43.5% and accompanied xerosis was found in 74.8% of the participants. No significant correlation was found betweenthe type of hospital unit worked and the presence of any OSD.

Another study from Poland by Kurpiewska et al. [8] showed that inflammatory OSD associated with xerosis and erythema seem to affect 80% of the cases. In this study, other professions such as hair stylists, cleaners, food service and textile workers were also included; the most prevalent OSD was found to be ICD and ACD [8]. Among these high risk occupations, healthcare professionals had the biggest exposure and latex gloves were blamed in 60% of hand dermatitis cases for midwives and 20% for hospital personnel [8]. Our current investigation showed a higher frequency (43.5%) for hand dermatitis in the nurse staff. These findings underline again the fact that hand dermatitis is a significant, ever-increasing OSD commonly seen in healthcare professionals.

In a different study from Taiwan, nursing staff was evaluated for the existence of hand eczema via a validated questionnaire, interestingly only 22% of the participants reported to have the clinical symptoms of hand eczema [9]. Working as a nurse for >10 years and being in charge of a special

care unit were significantly correlated with the development of hand dermatitis [9]. Similarly, the results of our current study revealed that longer nursing duration in years was associated with an increased risk of developing an OSD. We believe that longer exposure time to various allergens such as latex hand gloves, disinfectants and preservatives might have caused sensitization to these allergens thereby leading emergence of new-onset OSD. Similar to the findings of the study by Lan et al.[9] a self-reported survey showed a prevalence of20% for hand eczema among 1322 nurses worked in three different hospitals in China [10]. In this study the occurrence of hand eczema wasn't found to be significantly related to the gender and workplace which supports the results of our current investigation since we weren't able to find any correlation between the gender, the kind of hospital unit worked and the presence of any OSD.

DLQI is a basic, practical 10-item questionnaire which is developed to measure the psychological effects of different dermatological diseases on the patients [4]. In a recent study by Omrane et al. [11] it was shown that occupational dermatitis (OD) was most prevalently seen in nurses among all healthcare workers. In this study, a total of 37 OD cases were defined and the most prevalent OD was ACD [11]. The median DLQI was found to be 5 and about 1/3 of the affected subjects had an impaired DLQI [11]. In the current study, the median DLQI score was 4 and 76% of the participants had a score of ≥2, which means that the particular disease has an impact on the life quality of the patient [4,12].

Facial mask-induced acne seems to be an emerging problem among healthcare personnel and has drawn attention with the start of COVID-19 outbreak. In a study from Pakistan by Yaqoob et al. [13] during COVID-19 outbreak, acne was observed in 53.4% of the participants with female predominanceand development of facial acne was significantly correlated with the use of N-95 masks. Another recent study from Türkiye, which investigated the skin problems of COVID-19 ICU nursesrelated to the use ofPPE during COVID-19, showed that 90.24 % of the subjects were influenced by at least one skin disease induced by PPE [14]. The relevant skin problems included acne, contact urticaria, pressure sores and contact dermatitis [14]. In the current investigation, mask-induced or mask-exacerbated acne was found in only 23 (10%) participants, we believe that due to the decreased frequency of mask use in hospitals after COVID-19, facial acne cases might have declined in number.

Since the present study has a small sample size, further studies with larger sample sizes are needed to confirm our findings.

CONCLUSION

The present study shows that a significant proportion of nurse staff is affected by OSD and the extendednursing duration seems to be associated with higher risk of OSD development.

Author contribution

Study conception and design: EB; data collection: EB, HNB; draft manuscript preparation: EB, HNB. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Karatay University Non-Interventional Studies Ethics Committee (Protocol no. 2024/018, May 9 2024).

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] Smit HA, Burdorf A, Coenraads PJ. Prevalence of hand dermatitis in different occupations. Int J Epidemiol. 1993;22(2):288-293. https://doi.org/10.1093/ije/22.2.288

[2] Higgins CL, Palmer AM, Cahill JL, Nixon RL. Occupational skin disease among Australian healthcare workers: a retrospective analysis from an occupational dermatology clinic, 1993-2014. Contact Dermatitis. 2016;75(4):213-222. https://doi.org/10.1111/cod.12616

[3] Daye M, Cihan FG, Durduran Y. Evaluation of skin problems and dermatology life quality index in health care workers who use personal protection measures during COVID-19 pandemic. Dermatol Ther. 2020;33(6):e14346. https://doi.org/10.1111/dth.14346

[4] Finlay AY, Khan GK. Dermatology Life Quality Index (DLQI)--a simple practical measure for routine clinical use. Clin Exp Dermatol. 1994;19(3):210-216. https://doi.org/10.1111/j.1365-2230.1994.tb01167.x

[5] Diepgen TL, Coenraads PJ. The epidemiology of occupational contact dermatitis. Int Arch Occup Environ Health. 1999;72(8):496-506. https://doi.org/10.1007/s004200050407

[6] Kieć-Swierczyńska M, Krecisz B. Occupational skin diseases among the nurses in the region of Lódź. Int J Occup Med Environ Health. 2000;13(3):179-184.

[7] Lampel HP, Patel N, Boyse K, O'Brien SH, Zirwas MJ. Prevalence of hand dermatitis in inpatient nurses at a United States hospital. Dermatitis. 2007;18(3):140-142. https://doi.org/10.2310/6620.2007.06024

[8] Kurpiewska J, Liwkowicz J, Benczek K, Padlewska K. A survey of work-related skin diseases in different occupations in Poland. Int J Occup Saf Ergon. 2011;17(2):207-214. https://doi.org/10.1080/10803548.2011.11076880

[9] Lan CC, Feng WW, Lu YW, et al. Hand eczema among University Hospital nursing staff: identification of high-risk sector and impact on quality of life. Contact Dermatitis. 2008;59(5):301-306. https://doi.org/10.1111/j.1600-0536.2008.01439.x

[10] Zhang D, Zhang J, Sun S, Gao M, Tong A. Prevalence and risk factors of hand eczema in hospital-based nurses in northern China. Australas J Dermatol. 2018;59(3):e194-e197. https://doi.org/10.1111/ajd.12672

[11] Omrane A, Khedher A, Harrathi C, et al. Quality of Life of Healthcare Workers Suffering from Occupational Contact Dermatitis. Recent Adv Inflamm Allergy Drug Discov. 2022;15(1):44-51. https://doi.org/10.2174/1872213X14666210303155135

[12] Hongbo Y, Thomas CL, Harrison MA, Salek MS, Finlay AY. Translating the science of quality of life into practice: What do dermatology life quality index scores mean?. J Invest Dermatol. 2005;125(4):659-664. https://doi.org/10.1111/j.0022-202X.2005.23621.x

[13] Yaqoob S, Saleem A, Jarullah FA, Asif A, Essar MY, Emad S. Association of Acne with Face Mask in Healthcare Workers Amidst the COVID-19 Outbreak in Karachi, Pakistan. Clin Cosmet Investig Dermatol. 2021;14:1427-1433. https://doi.org/10.2147/CCID.S333221

[14] Altin L, Akbiyik A. Skin problems associated with using of personal protective equipment in COVID-19 intensive care units. Nurs Crit Care. 2023;28(6):985-995. https://doi.org/10.1111/nicc.12956

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ORIGINAL ARTICLE

8 years with laparoscopic liver surgery: a referral center experience

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ABSTRACT

Objective: Laparoscopic liver surgery (LLR) was first performed in 1992 and LLR began to be performed for many benign and malignant etiologies, especially hepatocellular carcinoma (HCC) and colorectal cancer metastases (CRC). Recent studies have shown that LLR has less bleeding, shorter hospital stay (LOS), faster recovery, and similar long-term oncologic outcomes compared to open surgery. This study aims to examine the results of LLR, which has been performed for 8 years in our institution, which is one of the referal centers in the field of hepatopancreaticobiliary surgery.

Methods: Twenty-nine patients who underwent LLR for malignant and benign reasons in our hospital between January 2016 and March 2024 were included in the study, and 416 patients who underwent open surgery, and laparoscopic ablation. Data were obtained retrospectively from the hospital registry system.

Results: 18 (62.1%) of the patients were male and the median age was 57 (41-62.5). 23 (79.31%) of the patients underwent surgery for malignant reasons. The most common indications for surgery were HCC (24.14%) and CRC (20.69%). Median blood loss was 200 (100-375) ml. Median LOS was three (3-5) days, and 30-day readmission rate was 3.45%. Clavian-Dindo ≥3 complication grade was seen in 13.79% of patients and no mortality was observed in any patient. R0 resection was achieved in 73.91% of patients. Disease recurrence developed in 56.52% of patients at a median follow-up of 22.1 (9.9-48.5) months. Of the patients who developed recurrence in the liver, recurrence developed at the surgical margin in 13.04%, and in other liver segments in 30.43%. Median overall survival was 48.5 months, and median recurrence-free survival was 21 months. 1-, 3-, and 5-year overall survival were 85%, 76%, and 48%, respectively, while 1- and 3-year recurrence-free survival were 71% and 34%, respectively.

Conclusion: In this study, it was shown that LLR is a safe alternative to traditional open surgery in terms of length of hospital stay, blood loss, recurrence rates, and survival rates in parallel with the literature, and that although the surgical margin was positive, recurrence developed mostly in other liver segments, and in some patients, no recurrence was observed despite positive margins.

Keywords: surgery, liver neoplasms, liver cancer, minimally invasive surgery, liver.

INTRODUCTION

Following the introduction of laparoscopic surgery hepatobiliary surgery in the 1990s, the first anatomical liver resection was performed in 1996 [1]. In the following years, laparoscopic surgery began to be used for more complex procedures due to less pain, faster recovery, cosmetic benefits, and increased experience. To ensure appropriate patient selection, standardization of surgical techniques, and improvement of outcomes, the first International Laparoscopic Liver Surgery Consensus Meeting was held in Louisville in 2008 [2]. Subsequently, the Moriaka consensus meeting in 2014 and the Southampton consensus meeting in 2017 established the standards for laparoscopic liver surgery (LLR) [3,4]. The indications of LLR is varied from benign liver diseases such as focal nodular hyperplasia (FNH) to malignant diseases, including hepatocellular carcinoma (HCC) and colorectal cancer metastases (CRC). In appropriate patients, LLR may result in less intraoperative bleeding, shorter hospital stay, faster recovery with similar long-term oncologic outcomes compared to open surgery [5-8]. Additionally, studies have shown that in cirrhotic patients, there is a lower incidence of postoperative ascites and liver failure, along with earlier initiation of adjuvant chemotherapy, which results in improved survival outcomes [9,10].

Our center is one of the referal centers in Turkey for hepatobiliary surgery. The living donor liver transplantation program was initiated in our institution in 2015, and LLR was performed for the first time in 2016. The aim of this study is to examine the outcomes of LLR procedures performed in our department over the past eight years for benign and malignant conditions.

PATIENTS AND METHODS

Patient Selection

Patients who applied our department between January 2016 and March 2024, and underwent LLR were included in the study. A total of 445 liver surgeries were performed, 29 (6.52%) of which were LLR. The exclusion criteria were as follows: <18 years old, open liver resections, laparoscopic and open microwave ablations (MWA), and patients in whom LLR was added to the primary surgery due to invasion of adjacent organs into the liver. Flow chart is shown in Figure 1.

Demographic, Clinical and Laboratory Features

Patients' demographic, clinical and laboratory characteristics were recorded retrospectively from hospital records. History of previous abdominal surgery, type and location of hepatic lesion, and imaging findings were evaluated. The operative findings, pathological results, surgical margin, postoperative findings, complications, blood replacement status, and length of hospital stay (LOS) were examined from the hospital records. Resection marjin was determined by histopathological analysis. In the postoperative follow-up, recurrence at the resection site, recurrence in another segment, or recurrence in another organ were evaluated separately.

Scores and Definitions

Charlson Comorbidity Index (CCI) is an accurate, easy, readily applicable, and a widely used score to calculate the mortality risk from comorbid diseases [11]. It predicts the 1-year mortality by weighting the comorbid diseases from 1 to 6, and the result is given by the total sum of the weights. The diseases included in this scoring system are as follows: myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, dementia, chronic obstructive pulmonary disease, connective tissue disease, peptic ulcer disease, liver disease, diabetes mellitus, hemiplegia, moderate to severe chronic kidney disease, solid tumor, leukemia, and lymphoma. The higher the CCI is, the higher the risk of mortality, and the severity of the comorbidities [11].

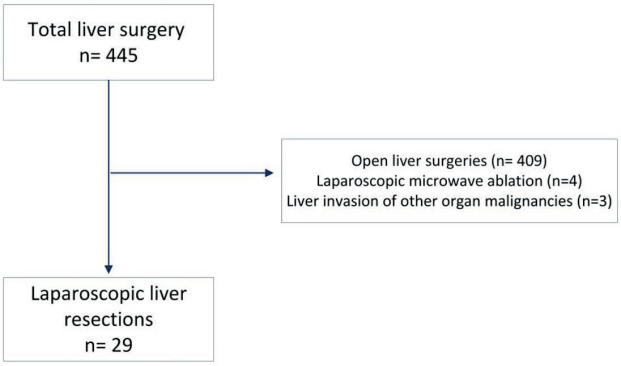

Figure 1. Flow chart

The American Society of Anesthesiologists (ASA) Score is the physical status classification system developed to predict the operative risk based on six classes (I-VI) [12]. I—a normal, healthy patient, II—a patient with mild systemic illness, III—a patient with severe systemic illness, IV—a patient with a severe systemic illness that is a constant threat to life, V—Moribund patient who is not expected to survive without the surgery, VI—a declared brain-dead patient whose organs are being removed for donor purposes.

Clavien-Dindo Classification is a widely used tool to evaluate postoperative complications (morbidity and mortality) [13,14]. The Clavien-Dindo Classification ranges from Grade I to Grade V as follows:

Grade I is any deviation from the normal recovery period after surgery without any need for a pharmacological or invasive procedure,

Grade II is any deviation from the normal recovery period after surgery requiring pharmacological treatment,

Grade III is any deviation from the normal recovery period after surgery requiring surgical, endoscopic, or radiological procedure (IIIa procedure not under general anesthesia; IIIb procedure under general anesthesia),

Grade IV is any deviation from the normal recovery period after surgery with life-threatening complications,

Grade V is the death of the patient after surgery.

The Iwate criteria is a surgical difficulty scoring system that was shown to be associated with operation time, estimated blood loss, open conversion rate, major complication rates, liver failure, and in-hospital death for minimally invasive liver surgery [15]. The total score is scored from 0 to 12 and is divided into 4 difficulty levels: low (0-3), intermediate (4-6), advanced (7-9), and expert (10-12). Variables were categorized into 6 groups: tumor location (1-5 points), hepatic resection type (0-4 points), tumor size (0-1 points), proximity to major blood vessels (0-1 points), Child-Pugh score (0-1 points), and hand-assisted/hybrid resection (0-1 points).

Brisbane 2000 terminology was used to describe tumor locations and surgical area. Couinaud's segments II, III, IVb, V, and VI were defined as anterolateral segments, segments I, IVa, VII, and VIII were defined as posterosuperior segments, segment II, and III were defined as left lateral sections [16].

Surgical Technique

Trocar sites are shown in Figure 2. After creating pneumoperitoneum, resection was performed under 12 mmHg pressure. Pringle maneuver preparation was performed through 5th trocar using tape and an 18-number chest tube in patients who were thought to have a risk of bleeding, were close to the portal hilus, had an Iwate score of 4 and above, and had fragile liver tissue secondary to hepatosteatosis. However, the Pringle maneuver was not performed in every patient who underwent Pringle preparation. In patients who underwent Pringle maneuver, a maximum of 3 cycles of 15 minutes of clamping and 10 minutes of unclamping were performed. The Pringle maneuver has been used as a method to reduce bleeding in open surgeries for many years. It is also used in laparoscopic liver surgery as a technique to reduce both bleeding and transfusion requirements. Other benefits include a clearer operational field with an improved visualization of

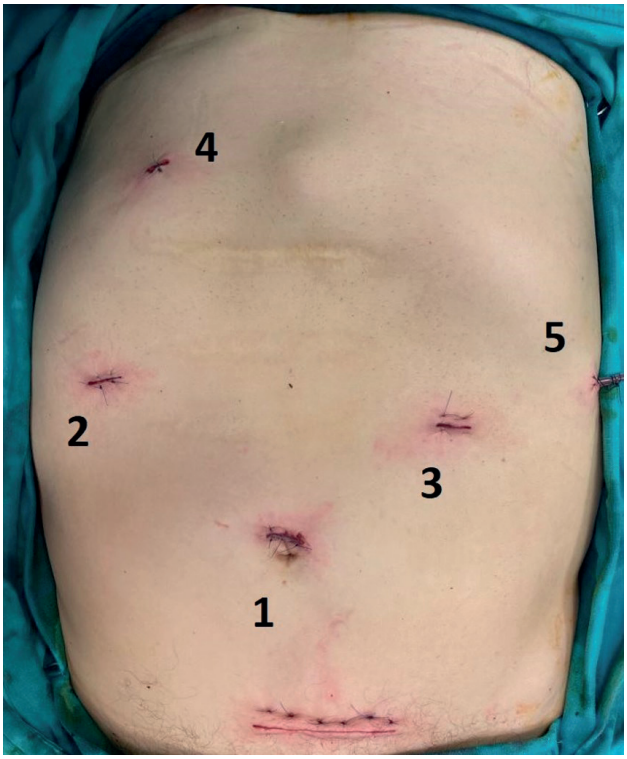

Figure 2. Trocar sites for laparoscopic liver resections **1.** Camera port **2-3.** Operation ports **4.** Asistance port **5.** Pringle maneuver port

intrahepatic vascular and biliary structures by the surgeon during the procedure [17]. Intraoperative laparoscopic ultrasonography was performed on all patients to evaluate the entire liver parenchyma. Likewise, resection margins and degree of proximity to major vessels were determined ultrasonographically. Hepatic transections were performed using a laparoscopic ligasureTM vessel sealing system (Medtronic, Minneapolis, USA) and an ultrasonic dissector (CUSA ExcelTM; Integra Lifesciences Corporation, Plainsboro, New Jersey, USA). Bipolar coagulation was used for minor bleeding and larger structures were controlled with endoclips, endoloop, and endoscopic linear staplers. Anatomic resections were performed by glissonian approach [18]. Nonanatomic resections were performed to provide safe surgical margins. If necessary, a Jackson-Pratt drain was placed in the surgical field from the 5th trocar (Figure 2). The specimen was extracted using a plastic bag through an additional 5-8 cm pfannenstiel incision.

Statistical Analysis

IBM SPSS Statistics for Windows, version 26.0 (SPSS Inc, Chicago, IL, USA) was used to perform the statistical analyses. The Mann–Whitney U test was used and continuous variables were presented as median and IQR values. The categorical variables were presented as frequency values. For categorical variables, chi-squared or Fischer's test was used when appropriate. Correlation analysis between variables was performed using the Spearman test for non-parametric variables. Correlation was determined using ρ (rho) value. Overall survival (OS) and recurrence free survival (RFS) were estimated using Kaplan-Meiers's analysis. A p-value < 0.05 was accepted as statistically significant.

RESULTS

Of a total of 29 patients who underwent LRR, eighteen (62.1%) were male and the median age was 57 years (41-62.5). Median body mass index (BMI) was 26 (23.69-29.2). While three (10.3%) of the patients were ASA III, the others were ASA I and II. Median CCI was six (3-8) and nine (31.03%) of the patients had a history of previous abdominal surgery. Twenty three LLR were for malignancy, including HCC (n=6), mix type (HCC+cholangiocarcinoma, one), CRC (n=6), malign melanoma metastases (n=4), lung cancer metastases (n=2), and one patient for each breast cancer metastases, gall bladder cancer, gastric cancer metastases, and testicular cancer metastases. Six LLR were for benign diseases, including hemangioma (n=2), and one patient for each hepatic adenoma, benign vascular neoplasia, biliary cyst adenoma, hepatolithiasis. Of the metastatic lesions, five (33.33%) were synchronous and ten (66.66%) were metachronous metastases. Cirrhosis was present in three (10.3%) patients. When maximum tumor sizes were examined, the median tumor diameter was 3.4 (2-4.95) cm. There were multifocal tumors in three (13.04%) patients. In five (17.24%) patients, the mass was close to major vessels. When the surgeries were examined in terms of difficulty levels, the median IWATE score was found four (2-5). It was determined that 12 (41.38%) patients had low difficulty level, 15 (51.72%) patients had intermediate difficulty level, and two (6.9%) patients had advanced difficulty level. Patients characteristics, tumor locations, and laboratory findings are summarized in Table 1.

Intraoperative and Postoperative Outcomes

Surgery was performed mostly on segments 2, 3 and 6. Table 2 summarizes the surgeries performed. Three patients (10.4%) had combined precedures for colorectal cancer (left lateral sectionectomy+ right lobe MWA+ open right hemicolectomy, left lateral sectionectomy+ laparoscopic left hemicolectomy or segment 2 metastasectomy+ laparoscopic left hemicolectomy). Two patients underwent two segments liver resections (segment 3 and 5).

Median operation time was 150 (120-197.5) minutes. Median estimated blood loss was 200 (100-375) mL. Four patients (13.79%) required intraoperative blood transfusion and the median transfusion rate was 400 (400-700) mL. Intraoperative total fluid transfusion rate was median 2000 (1450-2450) mL. Pringle maneuver was applied to five (17.24%) patients. The median pringle time in these patients was 20 (17.5-31) minutes. Two (6.9%) patients underwent conversion to open surgery, one for controlling bleeding during segment 8 resection, and the other because exposure could not be achieved during laparoscopic right hemicolectomy after laparoscopic left lateral sectionectomy+MWA. The median LOS was three (3-5) days. Six (20.69%) patients required postoperative intensive care unit (ICU) admission, and the median ICU stay was 1.5 (1-

Table 1. Demographic, clinical, and laboratory features

	Laparoscopic Liver Resection (n=29)
Sex (n,%)	
Male	18 (62.1)
Female	11 (37.9)
Age, median (IQR), years	57 (41-62.5)
Body mass index, median (IQR), kg/m^2	Med
Association of Anesthesiologist Classification, n (%)	
I: healthy	10 (34.5)
II: mild systemic disease	16 (55.2)
III: severe systemic disease	3 (10.3)
Charlson Comorbidity Index, median (IQR)	6 (3-8)
Previous abdominal surgery, n (%)	9 (31.03)
Neoadjuvant systemic theraphy, n (%)	12 (41.38)
Surgical etiology, n (%)	
Benign	6 (20.69)
Hemangioma	2 (6.9)
Adenoma	1 (3.45)
Benign vascular neoplasia	1 (3.45)
Biliary cyst adenoma	1 (3.45)
Hepatolithiasis	1 (3.45)
Malign	23 (79.31)
Primary	8 (27.59)
Hepatocellular carcinoma	6 (20.69)
Mixt type carcinoma (cholangio+hepatocellular)	1 (3.45)
Gall bladder carcinoma	1 (3.45)
Secondary	15 (51.72)
Colorectal cancer	6 (20.69)
Malign malenoma	4 (13.79)
Lung cancer	2 (6.90)
Breast cancer	1 (3.45)
Gastric cancer	1 (3.45)
Testicular cancer	1 (3.45)
Timing of metastases, n (%)	
Synchronous	5 (33.33)
Metachronous	10 (66.66)
Liver cirrhosis, n (%)	3 (10.3)
Maximum diameter of largest lesion (cm)*, median (IQR)	3.4 (2-4.95)
Proximity to major vessels, n(%)	5 (17.24)
Multifocal tumor**, n (%)	2 (8.70)
Number of tumor**, median (IQR)	1 (1-1)
Tumor locations, n (%)	
Segment II	4 (12.9)
Segment III	8 (25.8)
Segment II-III	4 (12.9)
Segment IVa	1 (3.23)
Segment IVb	3 (9.68)
Segment IVb-V	1 (3.23)
Segment IVb-V-gall bladder	1 (3.23)
Segment V	2 (6.45)
Segment VI	5 (16.13)
Segment V-VI	1 (3.23)
Segment VII	0
Segment VIII	1 (3.23)
IWATE score, median (IQR)	4 (2-5)
0-3 (low), n (%)	12 (41.38)
4-6 (intermediate), n (%)	15 (51.72)
7-9 (advanced), n (%)	2 (6.90)
10-12 (expert), n (%)	0

2) days. 30-day hospital readmission occurred in one (3.45%) patient and 90-day readmission occurred in four (13.79%) patients. Major adverse events (C-D ≥3a) were seen in 4 (13.79%) patients. One of them was treated with percutaneous drainage due to pleural effusion, one with endoscopic retrograde cholangiopancreatography (ERCP) + percutaneous drainage due to bile leakage, and two with percutaneous drainage due to postoperative fluid collection. Pathological examination results of 23 patients who underwent surgery due to malignancy revealed that surgical margins were negative (R0) in 17 (73.91%) patients and positive (R1+R2) in six (26.09%) patients. Surgical characteristics and outcomes are shown in Table 3.

Oncologic Outcomes and Recurrence

Of the 29 patients who underwent LLR, 23 were operated for malignant reasons. The median length of follow-up (including patients who died in postoperative period) was 22.1 (9.9-48.5) months. Disease recurrence was observed in 13 (56.52%) patients during postoperative follow-up. Recurrence was seen in the liver in 10 (43.48%)

Table 2. Surgial procedures

n, (%)	Laparoscopic liver resections (n=29)
Left lateral sectionectomy	6 (20.69)
+microwave ablation+open right hemicolectomy	1 (3.45)
+laparoscopic left hemicolectomy	1 (3.45)
Segment 2 resection	3 (10.34)
+laparoscopic left hemicolectomy	1 (3.45)
Segment 3 resection	5 (17.24)
Segment 4a resection	1 (3.45)
Segment 4b resection	3 (10.34)
Segment 4b+5 resection	2 (6.90)
Segment 6 resection	5 (17.24)
Segment 5-6 resection	1 (3.45)
Segment 3 and 5 resection	2 (6.90)
Lung cancer metastases	1 (3.45)
Hepatocellular carcinoma	1 (3.45)
Segment 8 resection	1 (3.45)

patients and in the extrahepatic area in 7 (30.43%) patients (some patients had both hepatic and extrahepatic recurrence). When liver recurrences were analyzed, three (13.04%) patients developed recurrence at the surgical site, and 7 (30.43%) patients developed recurrence in the other segments of the liver. The median time between surgery and the first recurrence was 10 (5.5-19.5) months. (Table 3) Median OS was 48.5 months. 1-year OS is 85%, 3-year OS is 76%, and 5-year OS was 48%. Median RFS is 21 months. 1-year RFS is 71% and 3-year RFS is 34%. (Figure 3) As of August 2024, seven (30.43%) patients died. Of the alive patients, 10 (43.48%) of them are disease-free.

When the seven patients with positive surgical margins were examined, it was seen that no recurrence was observed in two patients, recurrence developed in the resection margin in only one patient, and recurrence developed in other segments of the liver in the remaining three patients during the follow-up period. Recurrence analysis according to positive surgical margins is shown in Table 4.

The affect of surgical experience on the results was determined by comparing the results of the first 15 and last 14 patients. Accordingly, no statistically significant difference was found between the two groups in terms of operative time, intraoperative bleeding, complications, surgical margins and recurrence (p=0.16; 0.32; 0.26; 0.32; 0.32, respectively), while the median hospital stay was significantly reduced (4 (3-5), and 3 (2-4) days, respectively; p=0.018).

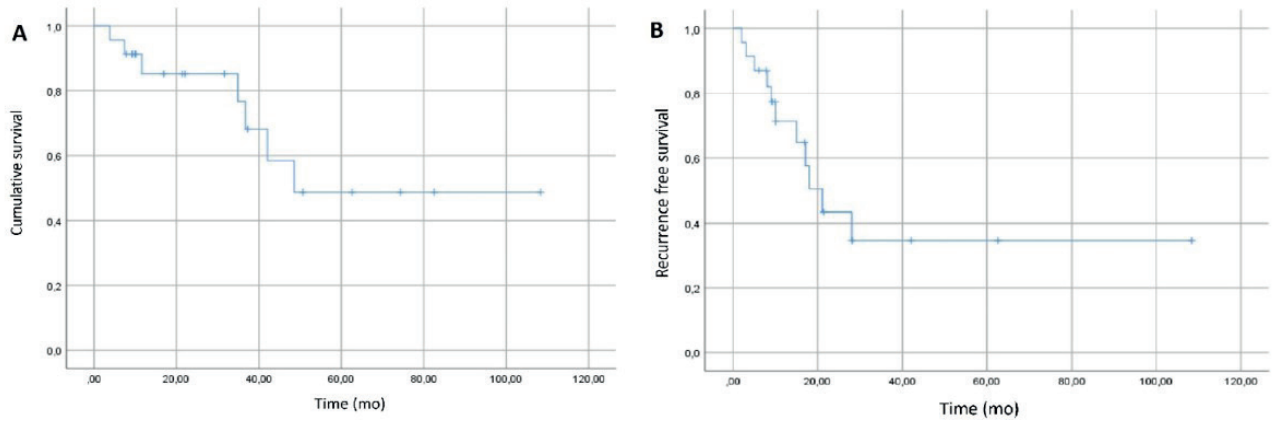

Figure 3. Kaplan-Meier Survival Curves, **A.** Overall survival **B.** Recurrence free survival

Table 3. Surgical characteristics and outcomes

	Laparoscopic Liver Resections (n=29)
Operation time (min), median (IQR)	150 (120-197.5)
Synchronous non-liver abdominal surgery, n (%)	3 (10.34)
Estimated blood loss (ml), median (IQR)	200 (100-375)
Intraoperative blood transfusion (patients), n (%)	4 (13.79)
amount of transfusion (ml), median (IQR)	400 (400-700)
Intraoperative total fluid transfusion (ml), median (IQR)	2000 (1450-2450)
Pringle maneuver performed, n (%)	5 (17.24)
Duration of pringle maneuver (min), median (IQR)	20 (17.5-31)
Conversion to open surgery, n (%)	2 (6.90)
Length of hospital stay (day), median (IQR)	3 (3-5)
Intensive care unit admission (patient), n (%)	6 (20.69)
if yes, length of admission (day), median (IQR)	1.5 (1-2)
Prolonged admission (>10 days), n (%)	1 (3.45)
30-days readmission, n (%)	1 (3.45)
30-days return to operating room, n (%)	0
90-days readmission, n (%)	4 (13.79)
Major adverse events (C-D ≥3a), n (%)	4 (13.79)
Clavien-Dindo classification, n (%)	
I	25 (86.21)
II	0
IIIa	4 (13.79)
IIIb	0
IV	0
V	0
Surgical margin*, n (%)	
Negative (R0)	17 (73.91)
Positive (R1+R2)	6 (26.09)
Total recurrence (patients)*, n (%)	13 (56.52)
Liver recurrence*, n (%)	10 (43.48)
Surgical site	3 (13.04)
Different segment	7 (30.43)
Extrahepatic recurrence*, n (%)	7 (30.43)
Time to first recurrence after surgery (month), median (IQR)	10 (5.5-19.5)
Overall survival* (month) median	48.5
Recurrence free survival* (month) median	21

C-D, Clavien-Dindo classification

*Evaluation was made only for malignant patients (n=23)

Correlation analysis

No correlation was found between the amount of intraoperative bleeding and IWATE score, complications, LOS, major vessel involvement and recurrence (p= 0.67; 0.14; 0.9; 0.09; 0.13, respectively). There was no correlation between the total amount of fluid transfusion and complications and LOS (p=0.25; 0.30, respectively). While no correlation was found between IWATE score and complications, surgical margin positivity and, recurrence (p=0.09; 0.09; 0.84; respectively), a positive direction, moderately strong, statistically significant correlation was found with the duration of surgery (p=0.008, ρ=0.49) (Figure 4).

Table 4. Surgical margin positivity and recurrence analysis

Primary pathology	Surgical site	Recurrence area	Time to recurrence after surgery	Current living situation
Hepatocellular carcinoma	Segment 3	No recurrence	49 months (disease free)	alive
Breast cancer metastases	Segment 4b	Surgical area (segment 4b)	18 months	alive
Lung cancer metastases	Segment 3 and 5	Segment 6 and 4-8	5 months	alive
Malign melanoma metastases	Segment 2	Segment 5-6 and 4-8	8 months	alive
Malign melanoma metastases	Segment 3	No recurrence	9 months (disease free)	alive
Testicular carcinoma metastases	Segment 4b	Multiple segments	2 months	exitus

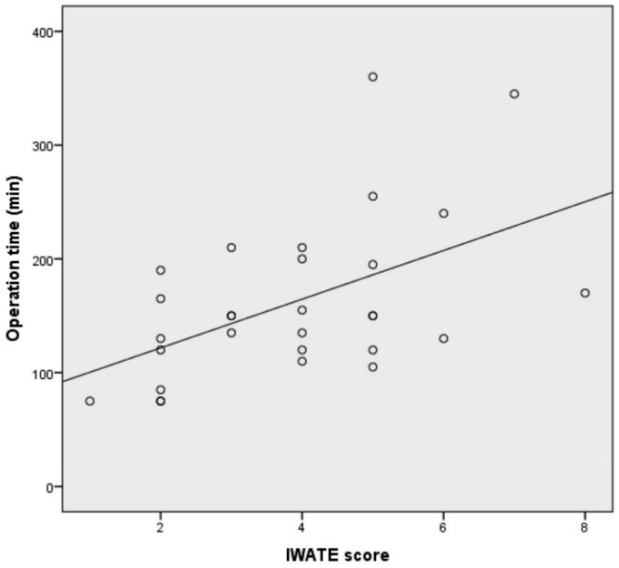

Figure 4. IWATE score and operating time correlation analysis

DISCUSSION

This study shows that LLR is an effective alternative to traditional open surgery in selected malignant and benign patients, with low complication rates, short hospital stays, and acceptable overall survival rates.

Following the introduction of laparoscopic techniques in many fields, the use of laparoscopy has also become increasingly common in hepatobiliary surgery. The first anatomical LLR was performed in 1996 as a left lateral sectionectomy (LLS) [1]. With experience, laparoscopy began to be defined as the standard treatment for LLS [4,8,19,20]. While LLR was performed in 2.3% of all liver surgeries in 2000, this rate increased to 7.5% have malignant causes [5]. According to a meta-analysis, 75% of malignant patients are HCC and CRC [21]. In our study, 6.52% of all liver surgeries were LLR and 79.31% of the indications were malignancies. Additionally, 56.52% of all malignancies in our study were CRC and HCC.

When the advantages of LLR were examined, the previous studies showed that there were fewer wound complications, less amount of bleeding, shorter surgery time with similar oncological results compared to open liver surgery [5-7]. It was also shown that there was less postoperative ascites and liver failure in the cirrhotic patient group [9]. In addition, laparoscopy is accepted as a surgical method that can be used safely in donor hepatectomy surgeries [22,23]. In a study conducted on liver transplant patients, 60% of the patients experienced wound problems such as wound tension, paresthesia, and hypertrophic scarring in the first year after surgery, and these problems continued in the long term in 35% of the patients [24]. Additionally, the incidence of incisional hernia after open liver surgery has been reported to be approximately 30% [25,26]. The incidence of surgical site infection and wound complications after LLR is very low [27]. In our study, similar to the literature, no surgical site infection or incisional hernia was observed in any patient.

Another benefit of LLR is less bleeding and less need for blood transfusion compared to open surgery. In the study by Bagante et al., where they examined the results of 2542 open hepatectomy and 612 LLR patients, it was observed that there was a statistically significant less need for blood transfusion and pringle maneuver in the LLR group (7.5% vs. 21.3%, $p<0.001$; 11.4% vs. 29.3%, $p<0.001$, respectively) [5]. In the same study, bile leakage occurred in 2.9% of the LLR group compared to 10.3% in the open surgery group ($p<0.001$). A meta-analysis of 9000 patients showed less estimated blood loss, and less blood transfusion, (322 ml vs 572 ml, $p<0.001$; 4.02% vs 9.57%, $p<0.001$; respectively) [21]. In contrast, in the randomized controlled "Orange II Plus study", one of the most recent studies in the literature comparing the results of laparoscopic and open major hemihepatectomies in 16 centers from Europe, the median blood loss

was reported to be similar in LRR and open surgery groups (450 [300-775] mL, and 450 [300-785] mL, respectively) (p=0.79) [28]. According to the results of the randomized controlled "LapOpHuva study" comparing laparoscopic and open surgery in patients with CRC liver metastasis, median operative time was 120 (90-180) minutes, median blood loss was 100 (50-300) ml, blood transfusion rate was 4.2%, pringle maneuver was performed in 30.2% of patients, and clamping time median was 15 (8-20) minutes [29]. In a recent study of 1258 patients in which the results of low risk patients were examined by expert surgeons and the benchmark criteria for LLR were determined, the operation time was defined as median of 200 (113-288) minutes, estimated blood loss as median of 100 (0-225) mL, intraoperative blood transfusion rate as 4.4%, and open conversion rate as 4.8% [30]. Similarly in our study, median of 200 (100-375) ml intraoperative blood loss occurred, but the need for blood transfusion was slightly higher (13.79%), and a median of 400 (400-700) ml blood transfusion was required. Consistent with the literature, pringle maneuver was performed in 17.24% of patients and median clamping time was calculated as 20 (17.5-31) min. However, the surgery time was slightly longer (median 150 (120-197.5) min), but conversion to open rate occurred in only 2 patients (6.9%).

In laparoscopic surgeries, the postoperative inflammatory response is less, thus the physical impact of the surgery is reduced and faster recovery is observed [31,32]. For this reason, the LOS is shorter than open surgery. In a study examining the quality of life after LLR, the median LOS for LLR was 2.2 (1.88-2.54) days, while it was 4 (3.71-4.29) days in open surgery (p<0.001). In the same study, body pain in the first month after surgery was significantly lower in the LLR group (p=0.003) [20]. In another study, median LOS was reported to be 3 (2-5) days in the LLR group while it was 6 (5-8) days in the open surgery group (p<0.001). The readmission rate was 7.5% for LLR [5]. A meta-analysis showed that LOS was shorter in both minor and major hepatectomy groups compared to open surgery (8.28±4.49 vs 13.54±8.8 days, p<0.001; 8.3±4.28 vs 16.67±8.3 days, p<0.001, respectively) [21]. In the "Orange II Plus study," LOS was reported as median 5 (4-7) days in the laparoscopy arm and 6 (5-7) days in the open surgery arm (p = 0.002). In the same study, readmission rate was reported as 13.3% [28]. In the benchmark study by Goh et al., median LOS was reported as 5 (4-7) days, 30-day readmission as 2.5% [30]. Our study showed a similar median LOS of 3 (3-5) days, with comparable 30-day and 90-day readmission rates (3.45%, 13.79%; respectively).

When the results of LLR were examined from an oncological perspective, it was stated that the resection margin and R0 resection rate were similar to LLR and open surgery [21]. In a recent anatomical liver resection study, the R0 resection rate in patients who underwent LLR was 77.9%, the R1-R2 resection rate, the recurrence rate, and the liver only recurrence rate were 19.1%, 48.5%, and 26.5%, respectively [28]. In the LapOpHuva study, R0 resection margin was obtained in 95.8% of patients, recurrence was seen in 67.7% of patients, and liver only recurrence was seen in 15.6% of patients [29]. According to the long-term results of the Oslo-Comet trial, recurrence developed in 67% of patients and liver only recurrence was observed in 33.75% of patients [33]. In the study by Goh et al., the R1 resection rate was determined as 4.5% [30]. In this current study, we showed that the R0 resection rate for malignant patients was 73.91%, while recurrence developed in 56.52% of patients during the follow-up period, and liver recurrence was seen in 43.48% of patients. When liver recurrences were examined, 13.04% recurrence was observed in the surgical site and 30.43% was observed in other segments. We attribute our R1-R2 resection rate being slightly higher than the literature to the fact that we have not yet completed the learning curve. Despite this, the overall recurrence and intrahepatic recurrence sites seen in patients were comparable to the literature.

Previous studies demonstrated the overall morbidity after LLR as 11 to 48%, and 30-day mortality is 0-2% [5,21,27,34]. The Orange II Plus study reported a 90-day liver-specific morbidity as 13.9% in the LLR group [28]. In the LapOpHuva study, the C-D≥3a complication rate was 6.25%, while 1-, 3-, and 5-year OS rates were 92.5%, 71.5%, and 49.3%, and 1-, 3-, and 5-year RFS were 72.7%, 33.5%, and 22.7%, respectively [29]. According to the long-term results of the Oslo-Comet trial, 1-, 3-, and 5- year OS rates were reported as 96.6%, 71.4%, 54.1%, and 1-, 3-, and 5- year RFS rates were reported as 55.5%, 35.9%, 29.7%, respectively. According to univariable analysis, there was no relationship between R1 (<1mm) resection and

RFS and OS, while in patients with R2 resection, RFS was shortened (p = 0.002) but OS did not change (p = 0.39) [33]. In terms of benchmark criteria, 90-day morbidity was reported as 13.8% with a 90-day mortality as 0.2% [30]. In our study, overall morbidity was shown to be 13.79% and 90-day mortality was 0%. Additionally, in malignant patients, the median RFS was found 21 months, and the median OS was 48.5 months. 1-, 3-, and 5-years OS were 85%, 76%, and 48%, and 1- and 3-years RFS were 71% and 34%.

It is stated that there is a learning curve of about 40-60 surgeries for LLR [35]. Chua et al. reported the learning curve in their study involving approximately 28% minor, 21% major, and 50% minor+major hepatectomies, with a median of 43 (18-60) patients [36]. In addition to the number of surgeries, the degree of difficulty of the surgery also affects the results. Although various difficulty scoring systems have been used, the IWATE scoring system is the most commonly used for laparoscopic and robotic liver surgeries. This score was created by examining the Japanese cohort after the 2014 Morioka consensus meeting, and scoring system correlates with conversion to open surgery, morbidity level, in-hospital mortality, and liver failure [3,15,37,38]. The median Iwate score of the surgeries in our study was four (2-5) and 51.72% of the patients had intermediate difficulty. Unlike the literature, a correlation relationship with Iwate score was found only between the duration of surgery (p=0.008, ρ=0.49), but this relationship could not be demonstrated in other parameters. Since the total number of LLRs performed is 29, we still have not been able to complete the learning curve. When the results of the first 15 surgeries were compared with the last 14 surgeries, the only statistically significant difference was found in the LOS results (median four (3-5) days vs three (2-4) days, respectively, p=0.018). No statistically significant difference was found in other parameters. We believe the results will improve as the number of surgeries increases.

Our study has some limitations. First, the number of patients was quite limited. Only 6.59% of patients underwent laparoscopic surgery. We attribute this to the fact that our hospital is a referral center for hepatobiliary surgery, and the patient group consists mostly of difficult and redo patients. The other limitation was that it was a retrospective and single center study, and the results of LLR could not be compared with the results of open surgery. In further studies, the results of 409 open hepatectomy patients not included in this study can be compared with the results of 29 laparoscopic liver surgeries using the propensity score match analysis method.

CONCLUSION

LLR is a safe and feasible alternative to traditional open surgery, in terms of hospital stay, blood loss, recurrence rates, and survival rates. In this era where minimally invasive surgery is evolving into robotic surgeries, further studies comparing the results of robotic and laparoscopic liver surgery are needed.

Author contribution

Study conception and design: HAD, ABD; data collection: HAD, DD; analysis and interpretation of results: HAD, DD, OC, ABD; draft manuscript preparation: HAD, OC. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Hacettepe University Health Sciences Research Ethics Committee (Protocol no. SBA 24/551 // 21.05.2024).

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] Azagra JS, Goergen M, Gilbart E, Jacobs D. Laparoscopic anatomical (hepatic) left lateral segmentectomy-technical aspects. Surg Endosc 1996;10(7):758-61. https://doi.org/10.1007/BF00193052

[2] Buell JF, Cherqui D, Geller DA, et al. The international position on laparoscopic liver surgery: The Louisville Statement, 2008. Ann Surg 2009;250(5):825-30. https://doi.org/10.1097/sla.0b013e3181b3b2d8

[3] Wakabayashi G, Cherqui D, Geller DA, et al. Recommendations for laparoscopic liver resection: A report from the second international consensus conference held in Morioka. Annals of Surgery 2015;261(4):619-29. https://doi.org/10.1097/SLA.0000000000001184

[4] Abu Hilal M, Aldrighetti L, Dagher I, et al. The Southampton Consensus Guidelines for Laparoscopic Liver Surgery: From Indication to Implementation. Ann Surg 2018;268(1):11-8. https://doi.org/10.1097/SLA.0000000000002524

[5] Bagante F, Spolverato G, Strasberg SM, et al. Minimally Invasive vs. Open Hepatectomy: A Comparative Analysis of the National Surgical Quality Improvement Program Database. J Gastrointest Surg 2016;20(9):1608-17. https://doi.org/10.1007/s11605-016-3202-3

[6] Chen Q, Merath K, Bagante F, et al. A Comparison of Open and Minimally Invasive Surgery for Hepatic and Pancreatic Resections Among the Medicare Population. J Gastrointest Surg 2018;22(12):2088-96. https://doi.org/10.1007/s11605-018-3883-x

[7] Aloia TA, Zimmitti G, Conrad C, Gottumukalla V, Kopetz S, Vauthey JN. Return to intended oncologic treatment (RIOT): A novel metric for evaluating the quality of oncosurgical therapy for malignancy. J Surg Oncol 2014;110(2):107-14. https://doi.org/10.1002/jso.23626

[8] Fretland ÅA, Dagenborg VJ, Bjørnelv GMW, et al. Laparoscopic Versus Open Resection for Colorectal Liver Metastases: The OSLO-COMET Randomized Controlled Trial. Ann Surg 2018;267(2):199-207. https://doi.org/10.1097/SLA.0000000000002353

[9] Azoulay D, Ramos E, Casellas-Robert M, et al. Liver resection for hepatocellular carcinoma in patients with clinically significant portal hypertension. JHEP Rep 2020;3(1):100190. https://doi.org/10.1016/j.jhepr.2020.100190

[10] Tohme S, Goswami J, Han K, et al. Minimally Invasive Resection of Colorectal Cancer Liver Metastases Leads to an Earlier Initiation of Chemotherapy Compared to Open Surgery. J Gastrointest Surg 2015;19(12):2199-206. https://doi.org/10.1007/s11605-015-2962-5

[11] Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: Development and validation. J Chronic Dis 1987;40(5):373-83. https://doi.org/10.1016/0021-9681(87)90171-8

[12] Doyle DJ, Hendrix JM, Garmon EH. American Society of Anesthesiologists Classification. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2017. Available at: https://www.ncbi.nlm.nih.gov/books/NBK441940/

[13] Clavien P-A, Sanabria JR, Strasberg SM. Proposed classification of complications of surgery with examples of utility in cholecystectomy. Surgery 1992;111(5):518-26.

[14] Dindo D, Demartines N, Clavien PA. Classification of surgical complications: A new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg 2004;240(2):205-13. https://doi.org/10.1097/01.sla.0000133083.54934.ae

[15] Wakabayashi G. What has changed after the Morioka consensus conference 2014 on laparoscopic liver resection?. Hepatobiliary Surg Nutr 2016;5(4):281-9. https://doi.org/10.21037/hbsn.2016.03.03

[16] Strasberg S, Belghiti J, Clavien P-A, et al. The Brisbane 2000 terminology of liver anatomy and resections. Hpb 2000;2(3):333-9. https://doi.org/10.1016/S1365-182X(17)30755-4

[17] Mownah OA, Aroori S. The Pringle maneuver in the modern era: A review of techniques for hepatic inflow occlusion in minimally invasive liver resection. Ann Hepatobiliary Pancreat Surg 2023;27(2):131-40. https://doi.org/10.14701/ahbps.22-109

[18] Machado MAC, Surjan RC, Basseres T, Schadde E, Costa FP, Makdissi FF. The laparoscopic Glissonian approach is safe and efficient when compared with standard laparoscopic liver resection: Results of an observational study over 7 years. Surgery 2016;160(3):643-51. https://doi.org/10.1016/j.surg.2016.01.017

[19] Chang S, Laurent A, Tayar C, Karoui M, Cherqui D. Laparoscopy as a routine approach for left lateral sectionectomy. Br J Surg 2007;94(1):58-63. https://doi.org/10.1002/bjs.5562

[20] Fretland ÅA, Dagenborg VJ, Waaler Bjørnelv GM, et al. Quality of life from a randomized trial of laparoscopic or open liver resection for colorectal liver metastases. Br J Surg 2019;106(10):1372-80. https://doi.org/10.1002/bjs.11227

[21] Ciria R, Cherqui D, Geller DA, Briceno J, Wakabayashi G. Comparative Short-term Benefits of Laparoscopic Liver Resection: 9000 Cases and Climbing. Ann Surg 2016;263(4):761-77. https://doi.org/10.1097/SLA.0000000000001413

[22] Samstein B, Cherqui D. Pure laparoscopic donor hepatectomy: A nearly finished product. Am J Transplant 2024;24(2):160-1. https://doi.org/10.1016/j.ajt.2023.08.013

[23] Samstein B, Griesemer A, Cherqui D, et al. Fully laparoscopic left-sided donor hepatectomy is safe and associated with shorter hospital stay and earlier return to work: A comparative study. Liver Transpl 2015;21(6):768-73. https://doi.org/10.1002/lt.24116

[24] Society JLT. Registry by the Japanese Liver Transplantation Society [in Japanese]. Ishoku 2014;49:261.

[25] Nilsson JH, Strandberg Holka P, Sturesson C. Incisional hernia after open resections for colorectal liver metastases - incidence and risk factors. HPB (Oxford) 2016;18(5):436-41. https://doi.org/10.1016/j.hpb.2016.02.001

[26] Kayashima H, Maeda T, Harada N, et al. Risk factors for incisional hernia after hepatic resection for hepatocellular carcinoma in patients with liver cirrhosis. Surgery 2015;158(6):1669-75. https://doi.org/10.1016/j.surg.2015.06.001

[27] Sasaki A, Nitta H, Otsuka K, Takahara T, Nishizuka S, Wakabayashi G. Ten-year experience of totally laparoscopic liver resection in a single institution. Br J Surg 2009;96(3):274-9. https://doi.org/10.1002/bjs.6472

[28] Fichtinger RS, Aldrighetti LA, Abu Hilal M, et al. Laparoscopic Versus Open Hemihepatectomy: The ORANGE II PLUS Multicenter Randomized Controlled Trial. J Clin Oncol 2024;42(15):1799-809. https://doi.org/10.1200/JCO.23.01019

[29] Robles-Campos R, Lopez-Lopez V, Brusadin R, et al. Open versus minimally invasive liver surgery for colorectal liver metastases (LapOpHuva): A prospective randomized controlled trial. Surg Endosc 2019;33(12):3926-36. https://doi.org/10.1007/s00464-019-06679-0

[30] Goh BK, Han H-S, Chen K-H, et al. Defining global benchmarks for laparoscopic liver resections: An international multicenter study. Annals of Surgery 2023;277(4):e839-48.

[31] Simillis C, Constantinides VA, Tekkis PP, et al. Laparoscopic versus open hepatic resections for benign and malignant neoplasms--a meta-analysis. Surgery 2007;141(2):203-211. https://doi.org/10.1016/j.surg.2006.06.035

[32] Fretland AA, Sokolov A, Postriganova N, et al. Inflammatory Response After Laparoscopic Versus Open Resection of Colorectal Liver Metastases: Data From the Oslo-CoMet Trial. Medicine (Baltimore) 2015;94(42):e1786. https://doi.org/10.1097/MD.0000000000001786

[33] Aghayan DL, Kazaryan AM, Dagenborg VJ, et al. Long-Term Oncologic Outcomes After Laparoscopic Versus Open Resection for Colorectal Liver Metastases : A Randomized Trial. Ann Intern Med 2021;174(2):175-82. https://doi.org/10.7326/M20-4011

[34] Reddy SK, Pawlik TM, Zorzi D, et al. Simultaneous resections of colorectal cancer and synchronous liver metastases: A multi-institutional analysis. Ann Surg Oncol 2007;14(12):3481-91. https://doi.org/10.1245/s10434-007-9522-5

[35] Berardi G, Aghayan D, Fretland ÅA, et al. Multicentre analysis of the learning curve for laparoscopic liver resection of the posterosuperior segments. Br J Surg 2019;106(11):1512-22. https://doi.org/10.1002/bjs.11286

[36] Chua D, Syn N, Koh YX, Goh BKP. Learning curves in minimally invasive hepatectomy: Systematic review and meta-regression analysis. Br J Surg 2021;108(4):351-8. https://doi.org/10.1093/bjs/znaa118

[37] Tanaka S, Kawaguchi Y, Kubo S, et al. Validation of index-based IWATE criteria as an improved difficulty scoring system for laparoscopic liver resection. Surgery 2019;165(4):731-40. https://doi.org/10.1016/j.surg.2018.10.012

[38] Barron JO, Orabi D, Moro A, et al. Validation of the IWATE criteria as a laparoscopic liver resection difficulty score in a single North American cohort. Surg Endosc 2022;36(5):3601-9. https://doi.org/10.1007/s00464-021-08561-4

acta medica

ORIGINAL ARTICLE

"C-shaped" anterolateral thigh flap for stomal repair due to recurrence in patients with total laryngectomy

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ABSTRACT

Background: Stomal recurrence after total laryngectomy is one of the most challenging problems in head and neck surgery due to the complexity of its management. The tracheal opening remains deep and caudal after resection, creating a neo stoma that presenting a significant challenge for reconstructive surgery. With this study, we aimed to describe the design of the C-shaped ALT flap to show that it is the ideal design in such a difficult reconstruction.

Methods: Five patients who underwent a free ALT flap for defects in the anterior neck wall and airway after resection due to tumor recurrence adjacent to a permanent stoma between 2018-2020 were included in the study. Tumor resection was performed by the Otolaryngology team in all cases. Age, gender, cause of defect, ALT flap size, donor site closure method, number of perforators, ischemia time, flap survival, early complications, and postoperative tracheostomy use and postoperative quality of life assessment were reviewed.

Results: 5 male patients were included in the study and the mean age was 63.8. The mean duration between admission and recurrence after laryngectomy was 11.2 months. Flap survival was noted in all patients. The patients did not encounter complications such as tracheostomy-related dehiscence, discharge, fistula, cannulation difficulties due to flap collapse, and mediastinitis during their lifetime. In the follow-ups, one still alive, the mean survival time of the other three patients was four months. We found an average high score in our patients regarding functional scales in QLQ-H&N35 module.

Conclusions: The C-shaped ALT flap design provides ease of insertion into a tension-free tracheal-skin suture line and helps to reduce the rates of stoma-related complications and increase the quality of life of the patients.

Keywords: ALT flap, C- shaped, stoma recurrence, tracheostomy.

INTRODUCTION

Stomal recurrence after total laryngectomy is one of the most challenging problems in head and neck surgery due to the complexity of its management [1]. Patients with stomal recurrence have a poor prognosis and a short life expectancy. The prognosis of most patients who face this serious complication is poor with a mortality rate of 98 percent [2].

The limited number of cases results in less surgical experience and limited information in the literature. The tracheal opening remains deep and caudal after resection, creating a neo stoma that presenting a significant challenge for reconstructive surgery.

Before the discovery of free tissue transfers, mediastinal tracheostomy was the preferred method in these patients. This method led to very serious vascular complications [3]. Later; local flaps, deltopectoral flap [4], thoracoacromial flap, pectoralis major myocutaneous flap[5,6], inframammary artery perforator flap (IMAP) [7], latissimus dorsi myocutaneous flap [8], bipediculated chest flaps were attempted. With the increasing popularity of free tissue transfers, these options were replaced by radial forearm flap (RFFF) [9] and anterolateral thigh flap (ALT) [10]. Free tissue transfer offers many additional flap options and may therefore address reconstruction needs more specifically. For example, they can be easily manipulated and adapted to complex defects. Moreover, they provide a large, well-vascularized and pliable skin area. The Flaps used in this type of defect have some basic problems. Firstly, most patients receive adjuvant radiotherapy treatment after total laryngectomy. This may lead to flap-related postoperative complications due to issues regarding recipient vessel. Secondly, the deep and caudal placement of the tracheal stump flap design requires keeping the vertical height long. If the vertical length of the flap not maintained, it may cause tension in the tracheal-skin suture line, thus dehiscence. Thirdly, the very deep and caudal location of the defect increases its distance to the neck and thus to the recipient vessel. Therefore, the flap pedicle should be kept long. Another problem is that patients use a tracheal cannula in the postoperative period, which may result in collapsing the flap and cannulation problems. For this reasons, aforementioned problems should be taken into consideration when designing the flap. The ideal reconstructive method should provide vascularized soft tissue that can close the defect after resection, allow the tracheal opening to be sutured to the skin edges without tension, and stand against collapsing to avoid obstructing the airway. We believe the C-shaped design of the ALT flap addresses all these problems, which makes this specific design of ALT flap the ideal reconstruction for this challenging area.

In this study, we aimed to describe the C-shaped design, advantages of the free anterolateral thigh (ALT) flap and observe the effect of the neo stoma on patients' quality of life.

MATERIALS and METHODS

Patients who underwent a free ALT flap for defects in the anterior neck wall and airway after resection due to tumor recurrence adjacent to a permanent stoma between 2018-2020 were included in the study. The study was approved by the Institutional Review Board/Ethics Committee and written consents were obtained. Resection, as well as flap design and reconstruction surgery, were performed by the same otolaryngology and reconstructive surgery team in all cases.The free flap used in accordance with the defect was designed in a C shape (Figure 1).

Age, gender, etiology of defect, ALT flap size, donor site closure method, number of perforators, ischemia time, flap survival, early complications, and postoperative tracheostomy use were reviewed. (Table 1) We used the QLQ-H&N35 module designed for patients with head and neck tumors to evaluate patients' quality of life. Except for the patient who died in the early postoperative period, 4 patients completed the QLQ-H&N35 module on the 20th postoperative day.

RESULTS

5 male patients were included in the study and the mean age was 63.8.(range 39 to 87) The mean duration between admission and recurrence after laryngectomy was 11.2 months (range 6 to 20 months); The mean duration of hospitalization in the intensive care unit after surgery was 6 days (range 2 to 15 days), and the mean duration of

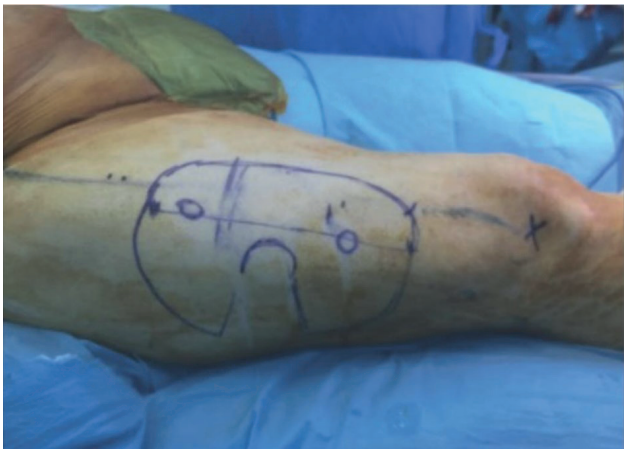

Figure 1. Preoperative planning

Table 1. Case series

	Age	Sex	Co-Morbidities	Time to Recurrence After Surgery	Postoperative Intensive Care Stay	Post Operative Hospital Stay	Post Operative Lifetime	Complications	Flap Size	Number of Perforators	Ischemia Time	Tumor Stage	Kt/Rt	Anastomosis
Patient 1	39	M	None	10 Months	2 Days	12 Days	7 Months	None	8X7 cm	2	55 Min	pT4aN0M0	Preop RT	Fasial a./v.
Patient 2	66	M	None	10 Months	3 Days	18 Days	1 Month	Dehiscence around the flap	15x10 cm	2	54 Min	pT3N0M0	Preop RT	Tca. / Ejv.
Patient 3	65	M	COPD	6 Months	3 Days	7 Days	Alive since 2020	Healing problem in the donor area	10x12 cm	1	40 Min	pT1N2aM0	Adjuvant KT+RT	Tca. / Ejv.
Patient 4	87	M	COPD, ICD, DM	20 Months	15 Days	15 Days	15 Days	Major bleeding	14x15 cm	2	91 Min	pT4aNXM0	Preop RT	Ima/Imv
Patient 5	62	M	CAD, DM, HT, Right Knee Amputation, Hypothyroidism	10 Months	7 Days	21 Days	4 Months	Dehiscence around the flap	19x9 cm	2	91 Min	pT4aNXM0	Preop RT	Tca. / Ejv.

COPD, Chronic Obstructive Pulmonary Disease; DM, Diabetes Mellitus; ICD, Ischemic Cerebrovascular Disease; HT , Hypertension; CAD, Coroner Artery Disease; Tca, Transverse Cervical Artery; Ejv, External juguler vein.

hospitalization was 14 days(range 4 to 21 days). Defect dimensions were measured as 13.2 x 10.6 cm on average (defect range 9X5 cm to 19x9 cm) in the intraoperative period. In one patient, the flap was harvested over a single perforator, while the flap was harvested over 2 perforators in other patients. Tumor invasion was observed in the innominate artery in the intraoperative period in one patient. The innominate artery was cleared of tumor by the department of Cardiovascular Surgery, but major bleeding occurred in the innominate artery within the first 24 hours after surgery. Later, the patient died as a result of complications related to major bleeding. In the follow-ups, one still alive, the mean survival time of the other three patients was four months.

Flap survival was noted in all patients. However, one patient died due to major bleeding in the early postoperative period. Minor dehiscence was detected at the flap margin in two patients in the postoperative period, probably due to radiotherapy damage in the native tissue, yet improvement was achieved with conservative follow-up within 6 weeks. Healing problem due to granulation tissue was observed in 1 patient in the donor area, and recovery was achieved with silver nitrate treatment. The patients did not encounter complications such as tracheostomy-related dehiscence, discharge, fistula, cannulation difficulties due to flap collapse, and mediastinitis during their lifetime.

Case report (Patient II):

A 66-year-old male patient was seen with a recurrent supraglottic squamous cell carcinoma after radiotherapy. The patient did not have any co-morbidities. He underwent total laryngectomy. A "C shape" ALT flap was used to reconstruct the anterior neck skin defect and the tracheostoma. The flap was based on two perforators and measured 15x10 cm. The flap was well perfused throughout. One week after surgery a small dehiscence appeared around the flap and healed very well with conservative treatment (Figure 2.,3.,4.).

In the postoperative EORTC QLQ - H&N35 module evaluation of the patient, functional scales were noted as role (42.36), emotional (50.64) and social (52.83).

The patient died of metastatic disease 1 months later.

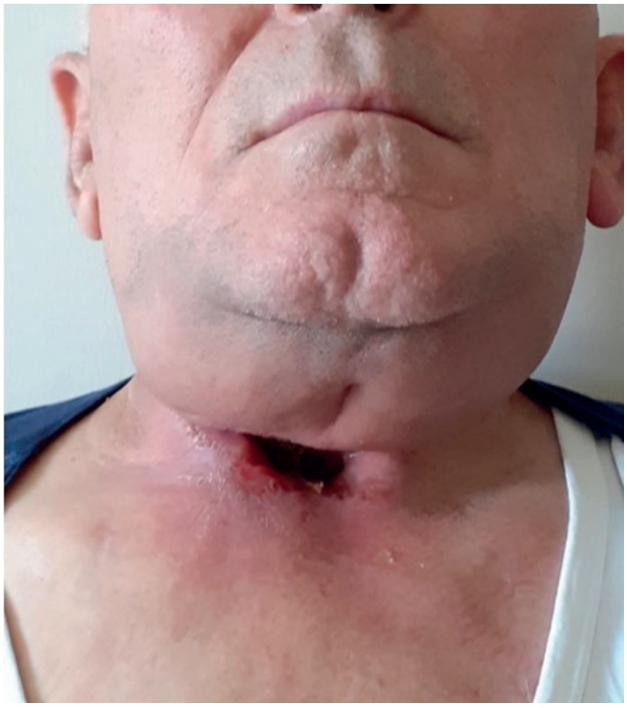

Figure 2. Preoperative photograph; Stoma recurrence after total laryngectomy

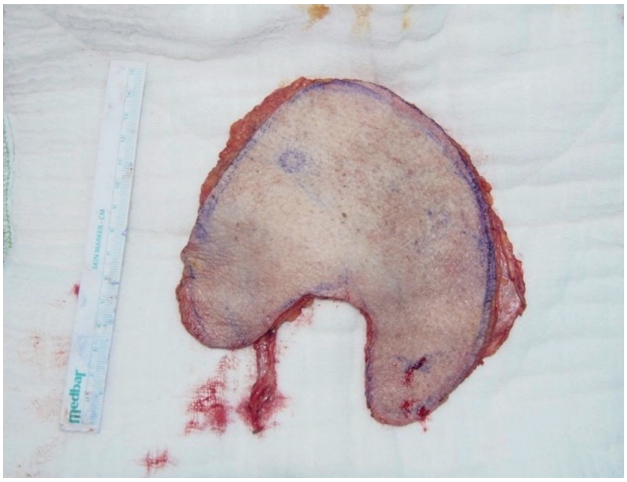

Figure 3. Intraoperative photograph; "C-shaped" ALT flap

DISCUSSION

In our series, the patients reconstructed with a C-shaped ALT flap due to stomal recurrence after laryngectomy, did not have tracheostomy-related problems in the postoperative period. None of our patients died due to reconstruction-related complications. Our patients were able to receive their treatment for their systemic metastases on time. Although the mortality was high, one of our patients continues to live in a healthy way. It was ensured that in such a mortal disease, patients' treatment for their malignancies was not delayed,

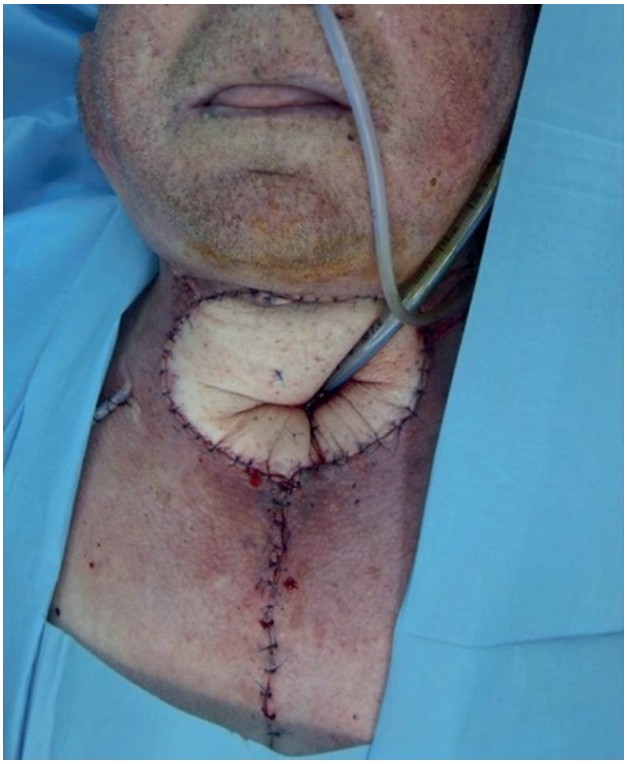

Figure 4. Postoperative photograph; "C-shaped" ALT flap for stoma repair after resection

and they did not suffer from tracheostomy-related problems with low quality of life in their probable short postoperative life. Our patients were discharged from the hospital in a short time and were able to continue their ordinary lives.

We think that the most important reason for our good reconstructive result is that our C-shaped flap is optimal for such defects. With the design of the C-shaped ALT flap, we adapt the inferior flap of the flap to the trachea after resection without tension. It can be adapted in a way that does not leave dead space for the defect by providing sufficient tissue volume at the superior of the flap. Thus, the irritation can be prevented by minimizing the contact of the postoperative tracheostomy cannula with the flap. The collapsed appearance can be avoided by providing sufficient volume of tissue. No matter how short the tracheal remnant is, the pedicle tension can be minimized by preferring the transverse cervical artery/vein anastomosis, which we used in most of our patients, while flap adaptation is performed without tension. In our view, design with 2 perforators, we prevented flap circulation disorders that may have occurred due to flap size. Concerning the difficult problem of reconstruction after resection of stomal recurrence after total laryngectomy, Caliceti et al. [10]

published a case report on the use of ALT flaps for this application. We argue that this flap, which is designed in the form of a tube, causes collapse over time and causes various cannulation difficulties. A brief comparison of flaps designed for stomal repair after resection due to recurrence in patients with total laryngectomy is provided. (Table 2).

We strongly believe that a C-shaped ALT is more suitable flap design than a tube-shaped ALT. In our experience, the tracheal mouth was easily adapted to the flap without tension thanks to the C-shaped design, and there was no collapsed appearance. We believe that the freedom in planning the dimensions of the part to be adapted to the tracheal residue, regardless of the extent of excision from the skin outside the stoma, provides a significant advantage in the design of this flap. The patients in our case series did not experience neo stoma-related complications during their lifetime.

The QLQ-H& N35incorporates seven multi-item scales that assess pain, swallowing, senses (taste and smell), speech, social eating, social contact, and sexuality. In addition, eleven single item assess problems with teeth, opening the mouth, dry mouth, sticky saliva, coughing, feeling ill, use of pain killers, nutritional supplements, or a feeding tube, weight loss and weight gain [11]. The functional scale scores are inverse: i.e., the higher the score, the better the function; on the symptom scales and independent items, the higher the score, the greater the difficulties or symptoms. It is found out that an average high score in our patients regarding functional scales: role (48.43), emotional (54.64) and social (57.43) and a low score on insomnia (15.64) and financial difficulties (25.76). In the study of Dinescu et al. [12] using the EORTC QLQ - H&N35 module for patients with total laryngectomy; they found that patients received low functional scores and a high score on the symptom scales and independent items.

Stomal recurrence is considered the most serious and fatal complication of laryngeal cancer. The highest cure rate in stomal recurrence after laryngectomy is achieved with aggressive radical surgery. In many series, the incidence of stoma recurrence ranges from 1.7% to 25%. However, since many articles count this complication among local complications, it is not possible to know the true value [13,14]. Stomal recurrence is commonly considered to be incurable. Even with aggressive surgical therapy and radio- therapy, these patients

Table 2. Comparison with other studies

	Flap Type	Survival After Surgery	Advantages	Disadvantages	Number of Cases
Withers EH et al.[6]	Pec. Major Flap	6 months	Easy to harvest; decrease the incidence of great vessel rupture, good option for salvage	Too bulky; 10% to 20% skin paddle necrosis; donor site contour deformity	1
Shinoda M et al.[8]	L. Dorsi Flap	3 years	Abundant vascularity, wide mobility, provides bulk to fill the dead space, easy to harvest	Not a first choice because of its more distant location	1
Yu P et al.[7]	IMAP	Mean 3.5 months	Thin and well- vascularized tissue; less bulky	Small size, unable to prevent vascular accidents	2
Cordeiro et al.[9]	RFFF	Not mentioned	Provides a large area of well-vascularized, pliable skin	Tension on the trachea–skin suture line because of limited size, unable to fill the dead space	1
Caliceti et al.[10]	Tubed ALT	22 months postoperatively alive	Low donor site morbidity, long and reliable pedicle. Possible chimeric design to fill dead space	Steep learning curve of perforator flaps; significant hair regrowth, low secretion clearance, collapsed appearance	1
Our Study	C-shaped ALT	Mean 4 months / 1 patient still alive since 2020	In addition to advantages of tubed ALT; no collapsed appearance, no neo stoma-related complications, tension free trachea-skin suture line	No additional disadvantages compared to the above tubed ALT (with no collapsed appearance)	5

Pec.Major Flap, Pectoralis Major Flap; L.Dorsi Flap, Latissimus Dorsi Flap ; IMAP , Internal Mammary Arter Perforator Flap ; RFFF , Radial Forearm Free Flap ; ALT , Anterolateral Thigh Flap.

usually succumb to respiratory obstruction or massive hemorrhage. Before the discovery of free tissue transfer, mediastinal tracheostomy was used as the standard reconstructive procedure after resection due to recurrence in patients with total laryngectomy. It was reported that wound dehiscence, mediastinitis, innominate artery erosion and vascular complications was not uncommon in patients after mediastinal tracheostomy also patient comfort is extremely poor [15]. Sisson (13) used mediastinal tracheostomy for reconstruction and achieved one of the best survival rates, with a 35% survival rate at 42 months for 75 stomal recurrences over 30-year period. Chemoradiotherapy protocols have not been shown to offer curative or palliative results to this extent. The mean survival time was calculated as 8.9 months in patients with stomal recurrence [16]. The mean survival time of 4 patients in our study was determined as 4 months and 1 patient is still alive, which is comparable with the literature. We think that inadequate resection margins due to proximity of the innominate artery in three of our patients resulted in close or positive surgical margins in the postoperative pathologic assessment, which we believe was the cause of such a high mortality rate in our series. All of the patients died due to the complications of metastatic disease. As the literature shows, we are aware of that the surgery performed on these patients was more palliative than cure. We found that these patients were protected from complications around the stoma in their short life span, thanks to this design, and their treatment is aimed at systemic disease.

Local flaps help to close defects of limited size. Thoracoacromial and deltopectoral flaps can be used for small defects, but distal parts may have circulation problems and have insufficient maneuverability. The pectoralis myocutaneous flap defined for this type of defects by Withers et al.6 provides substantial vascularized skin and muscle flaps and has been shown to reduce the incidence of vascular complications. We do not prefer the latissimus dorsi myocutaneous flap for such defects as described by Shinoda M et al. [8] due to its distant location. We believe that the internal mammary artery perforator flap (IMAP) for such defects as described by Yu P et al. [7] is thinner than other regional options, thus providing better aesthetic results than deltopectoral and pectoralis myocutaneous flaps. However, the size limits for safe removal of the IMAP flap have not been defined. Hence, it may be a better choice for small defects.

Cordeiro et al. [9] favored the radial forearm free flap (RFFF) for reconstruction in such patients because of its advantages such as flexibility, thinness, and long pedicle. Here, a repair was made for the skin defect and a hole was made in the middle of the flap and the trachea was placed. However, if the skin defect is large and/or the tracheal remnant is short, we believe that would result in tension in the trachea-skin suture line.

Wheatley et al. [17] later designed a tubular radial forearm flap mostly for tracheal reconstruction to reduce this suture tension. While the entire flap is used for tracheal reconstruction, the remaining skin defect is closed with the help of local myocutaneous flaps.

Low number of cases is the main limitation of our study. We attribute this to the fact that patients with stoma recurrence are generally deemed inoperative for various reasons by the oncology team, and the patients are referred directly to palliative treatment centers.

CONCLUSION

The ALT flap is a reliable and safe flap with a long pedicle, and the C-shaped design provides ease of insertion into a tension-free tracheal-skin suture line. Although the survival of patients with stomal recurrence after laryngectomy is very low due to metastatic disease, reconstruction with a C-shaped free ALT flap helps to reduce the rates of stoma-related complications and increase the quality of life of the patients. Future studies with larger patient cohorts and longer survival times are needed.

Author contribution

Study conception and design: BS, AHS, ÖS ; data collection: AHS, ÖS analysis and interpretation of results: BS, ZDA; draft manuscript preparation: AHS, ÖS All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Clinical Research Ethic Committee of Marmara University Facult of Medicine (Protocol no. 09.2024.922).

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] Mantravadi R, Katz AM, Skolnik EM, Becker S, Freehling DJ, Friedman M. Stomal recurrence. A critical analysis of risk factors. Arch Otolaryngol 1981;107(12):735-8. https://doi.org/10.1001/archotol.1981.00790480011003

[2] Oztürkcan S, Calli C, Dündar R, et al. Stomal recurrence after total laryngectomy: clinical and patological analysis of risk factors. Kulak Burun Bogaz Ihtis Derg 2009;19(3):146-50.

[3] Sisson GA, Straehley CJ, Johnson NE. Mediastinal dissection for recurrent cancer after laryngectomy. Laryngoscope 1962;72:1064-77. https://doi.org/10.1288/00005537-196208000-00008

[4] Portnoy WM, Arena S. Deltopectoral island flap. Otolaryngol Head Neck Surg 1994;111(1):63-9. https://doi.org/10.1177/019459989411100113

[5] Ariyan S. The pectoralis major myocutaneous flap. A versatile flap for reconstruction in the head and neck. Plast Reconstr Surg 1979;63(1):73-81. https://doi.org/10.1097/00006534-197901000-00012

[6] Withers EH, Davis JL, Lynch JB. Anterior mediastinal tracheostomy with a pectoralis major musculocutaneous flap. Plast Reconstr Surg 1981;67(3):381-5. https://doi.org/10.1097/00006534-198103000-00022

[7] Yu P, Roblin P, Chevray P. Internal mammary artery perforator (IMAP) flap for tracheostoma reconstruction. Head Neck 2006;28(8):723-9. https://doi.org/10.1002/hed.20386

[8] Shinoda M, Takagi I. Anterior mediastinal tracheostomy with a latissimus dorsi musculocutaneous flap. Br J Plast Surg 1992;45(2):160-2. https://doi.org/10.1016/0007-1226(92)90178-z

[9] Cordeiro PG, Mastorakos DP, Shaha AR. The radial forearm fasciocutaneous free-tissue transfer for tracheostomy reconstruction. Plast Reconstr Surg 1996;98(2):354-7. https://doi.org/10.1097/00006534-199608000-00024

[10] Caliceti U, Piccin O, Cavicchi O, Contedini F, Cipriani R. Anterolateral thigh free flap for tracheal reconstruction after parastomal recurrence. Head Neck 2009;31(8):1107-11. https://doi.org/10.1002/hed.20992

[11] Degboe A, Knight SL, Halling K, et al. Patients' experience of recurrent/metastatic head and neck squamous cell carcinoma and their perspective on the EORTC QLQ-C30 and QLQ-H&N35 questionnaires: a qualitative study. J Patient Rep Outcomes 2018;2:33. https://doi.org/10.1186/s41687-018-0060-7

[12] Dinescu FV, Tiple C, Chirilă M, Mureşan R, Drugan T, Cosgarea M. Evaluation of health-related quality of life with EORTC QLQ-C30 and QLQ-H&N35 in Romanian laryngeal cancer patients. Eur Arch Otorhinolaryngol 2016;273(9):2735-40. https://doi.org/10.1007/s00405-015-3809-0

[13] León X, Quer M, Burgués J, Abelló P, Vega M, de Andrés L. Prevention of stomal recurrence. Head Neck 1996;18(1):54-9. https://doi.org/c2cxzp

[14] Yotakis J, Davris S, Kontozoglou T, Adamopoulos G. Evaluation of risk factors for stomal recurrence after total laryngectomy. Clin Otolaryngol Allied Sci 1996;21(2):135-8. https://doi.org/10.1111/j.1365-2273.1996.tb01317.x

[15] Sisson GA, Bytell DE, Edison BD, Yeh S. Transsternal radical neck dissection for control of stomal recurrences--end results. Laryngoscope 1975;85(9):1504-10. https://doi.org/10.1288/00005537-197509000-00012

[16] Rubin J, Johnson JT, Myers EN. Stomal recurrence after laryngectomy: interrelated risk factor study. Otolaryngol Head Neck Surg 1990;103(5):805-12. https://doi.org/10.1177/019459989010300523

[17] Wheatley MJ, Meltzer TR, Cohen JI. Radial forearm free flap tracheal reconstruction after parastomal tumor resection. Plast Reconstr Surg 1998;101(5):1342-4. https://doi.org/10.1097/00006534-199804050-00030

acta medica

CASE REPORT

Daratumumab-associated varicella-zoster virus meningoencephalitis in relapsed refractory multiple myeloma

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ABSTRACT

Daratumumab is a widely-used monoclonal anti-CD38 antibody both in newly-diagnosed and relapsed refractory multiple myeloma. CD38 is expressed on the surface of NK, regulatory B, and T cells. Therefore, patients receiving the drug are prone to decreased immunity, especially against herpes virus infections. Varicella-zoster virus is one of the herpesviruses, and reinfection typically occurs in immunocompromised individuals, including multiple myeloma patients, by reactivation of endogenous latent infection within the sensory ganglia. This type of infection (herpes zoster) usually presents in a dermatomal skin area. Here, we report a patient who developed varicella-zoster virus meningoencephalitis under daratumumab treatment.

Keywords: daratumumab, herpes zoster infection, multiple myeloma, immunoglobulin, reactivation.

INTRODUCTION

Daratumumab, a human anti-CD38 antibody of the immunoglobulin (Ig)G1 isotype, is widely used in multiple myeloma (MM) and other plasma cell disorders for its antibody-dependent cellular cytotoxicity, as well as its Fc-dependent complement-dependent cytotoxicity and antibody-dependent cellular phagocytosis [1]. MM patients have a 14.8-fold risk of herpes zoster infections, which are the leading cause of infections in these patients, and daratumumab use may facilitate the development of herpes infections by disrupting immune responses via the depletion of natural killer (NK) cells and other CD38-expressing immune cells [1,2]. Here, we report an MM-diagnosed patient who developed varicella-zoster virus (VZV) meningoencephalitis under daratumumab treatment.

CASE PRESENTATION

A 68-year-old female patient was diagnosed with IgG lambda type MM in 2018. A bortezomib–lenalidomide–dexamethasone (VRd) regimen was initiated, together with acyclovir 800 mg/day twice a week for herpes prophylaxis. She refused to proceed with high-dose therapy with autologous stem cell rescue. After eight cycles of VRD treatment, she continued treatment with lenalidomide/dexamethasone for 4 years. After she relapsed, her treatment was switched to pomalidomide/dexamethasone as oral therapy to restrict hospitalization during the COVID-19 outbreak. Unfortunately, she had progressive disease, and a few months later, daratumumab monotherapy with dexamethasone was initiated, at 16 mg/kg weekly for 8 weeks. After 2 months, she was admitted to hospital because of new-onset confusion and fever. She did not have neck

stiffness. Hematological findings were as follows: leukocytes, 8720/mm3; neutrophils, 4850/mm3; lymphocytes, 2380/mm3; hemoglobin, 8.7 g/dL; and platelets, 43,000/mm3. Her IgG, IgM, and IgA levels were 41.55 g/L (6–15), 0.16 g/L (0.5–2.5), and 0.17 g/L (0.5–4), respectively. Lumbar puncture was performed. Direct microscopic examination detected 30 erythrocytes/mm3, 10 leukocytes/mm3, and no plasma cells, and Gram staining revealed no microorganisms. Cerebrospinal fluid analysis showed protein and glucose levels of 38 g/L (15–40) and 79 mg/dL (50–80), respectively. No bacteria were produced on culture. A rapid meningitis viral panel including herpes simplex virus type 1 and 2, human parechovirus, enterovirus, and VZV, and employing polymerase chain reaction, detected VZV positivity. In her serum, VZV IgG level was positive at 20.1 NovaTec units (NTU) (0–0.9); however, IgM level was negative at 0.15 NTU. Her HIV status was negative. On magnetic resonance imaging, bilateral high-signal foci were observed in the periventricular and supraventricular white matter on T2-FLAIR, some of which tended to coalesce in the subcortical area (Figure 1). Acyclovir treatment, intravenously at 10 mg/kg twice a day, was initiated and continued for 3 weeks. The patient never developed skin findings suggestive of VZV involvement before the central nervous system infection or during the treatment. She experienced significant benefits from the treatment and recovered without neurological sequelae.

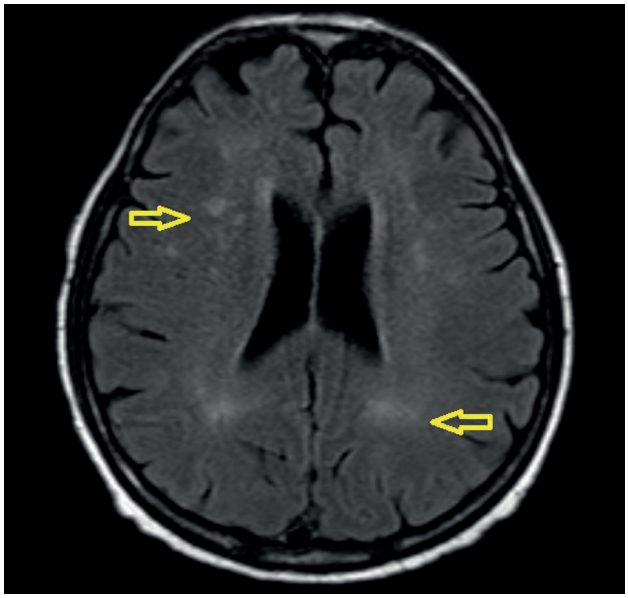

Figure 1. Bilateral high-signal foci in the periventricular and supraventricular white matter, some of which tended to coalesce in the subcortical area (arrows, FLAIR sequence MRI)

DISCUSSION

MM patients are prone to infections because of B cell dysfunction such as hypogammaglobulinemia, as well as abnormalities in T, dendritic, and NK cells. Daratumumab may reactivate viral infections by inhibiting CD38, which is expressed on the surface of NK, regulatory B, and T cells [2]. This is more prominent in MM patients heavily treated with prior lines. In addition, daratumumab is suggested to decrease CD4 T and NK cells and cause a low CD4/CD8 ratio, which makes the patient prone to viral reactivations [3]. Furthermore, limited data suggest that daratumumab penetrates the central nervous system (CNS), although this should be supported by extensive research. This may render the patient prone to the development of infections and reactivation of herpes virus in the CNS [4].

A meta-analysis by Vassilopoulos et al., examined the cumulative risk of infection in MM patients receiving anti-CD38 monoclonal antibody-based therapy and reported no increase in VZV infections due to daratumumab, despite an almost 30% higher risk of overall infections [5]. However, pharmacovigilance data from the Food and Drug Administration Adverse Events Reporting System report an increased risk of VZV reactivation when comparing patients receiving daratumumab with patients receiving other anti-myeloma treatments [6]. Drugs from other groups, such as proteasome inhibitors and/or dexamethasone, which are often used in combination with daratumumab, also increase this risk [5].

Our case was a relapsed refractory MM case who had previously received three lines of treatment. It should be emphasized that since dexamethasone was used together with daratumumab, the development of this infection should not be attributed to daratumumab alone. To our knowledge, our patient is the first case of daratumumab-associated herpes zoster meningoencephalitis reported in the literature. Thus, particular attention should be paid to the possibility of daratumumab causing viral infections in the CNS region, especially in heavily treated MM patients.

Author contribution

Study conception and design: AT, İY, AZB; data collection: AT, İY; analysis and interpretation of

results: AT, İY; draft manuscript preparation: AT. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

Since this is a case report, it does not require ethical committee approval. The informed consent was obtained from the patient for the participation and publication.

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] Nahi H, Chrobok M, Gran C, et al. Infectious complications and NK cell depletion following daratumumab treatment of Multiple Myeloma. PLoS One 2019;14(2):e0211927. https://doi.org/10.1371/journal.pone.0211927

[2] Blimark C, Holmberg E, Mellqvist UH, et al. Multiple myeloma and infections: A population-based study on 9253 multiple myeloma patients. Haematologica 2015;100(1):107-13. https://doi.org/10.3324/haematol.2014.107714

[3] Ferreira LM, Cerezer JL, Gehrcke M. Do cytomegalovirus infections affect the daratumumab treatment course in multiple myeloma patients? - Literature review. Hematol Transfus Cell Ther 2021;43(2):185-190. https://doi.org/10.1016/j.htct.2020.05.009

[4] Vercruyssen M, El Hachem G, Maerevoet, M. The Daratumumab Crosses the Blood Brain Barrier. Clinical Lymphoma Myeloma and Leukemia 2018;18:S289. https://doi.org/10.1016/j.clml.2018.07.229

[5] Vassilopoulos S, Vassilopoulos A, Kalligeros M, Shehadeh F, Mylonakis E. Cumulative Incidence and Relative Risk of Infection in Patients With Multiple Myeloma Treated With Anti-CD38 Monoclonal Antibody-Based Regimens: A Systematic Review and Meta-analysis. Open Forum Infect Dis 2022;9(11):ofac574. https://doi.org/10.1093/ofid/ofac574

[6] Burns EA, Ensor JE, Anand K, et al. Opportunistic infections in patients receiving daratumumab regimens for multiple myeloma (MM). Blood 2021;138(Suppl 1):4740. https://doi.org/10.1182/blood-2021-152068

acta medica

CASE REPORT

Femoral tunneled hemodialysis catheter insertion through subacutely occluded lower extremity central veins in patients with exhausted vascular access

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ABSTRACT

Maintaining venous access and catheter patency in patients undergoing hemodialysis through the central catheter is a dire necessity. When conventional venous accesses have been exhausted, unconventional venous access techniques have become rational options to create vascular access for hemodialysis. Herein, a case with exhausted venous access, that underwent tunneled dialysis catheter insertion through subacutely occluded lower extremity central veins was described.

Keywords: dialysis catheter, unconventional, vascular access, femoral, subacute occlusion.

INTRODUCTION

Although tunneled hemodialysis catheters (TDC) are considered inferior to arteriovenous fistulas in terms of infection and patency rates, they are widely used for hemodialysis. They are relatively easy to insert and immediately available to use. Therefore, central venous catheterization is one of the most common procedures performed in an interventional radiology unit [1,2]. Gradual exhaustion of venous access sites is a serious problem for patients dependent on hemodialysis. In case of exhausted upper extremity venous access, femoral TDCs are a reasonable option for vascular access, even though they have lower patency rates than upper extremity TDCs [3]. Also, when conventional vascular access creation attempts have failed, TDC insertion through acutely or chronically occluded central vessels can be considered [1,4]. Herein, we describe a case when all conventional vascular access creation attempts had failed, and a femoral TDC was inserted through subacutely occluded lower extremity central veins.

CASE REPORT

A 24-year-old female patient with a history of surgery due to persistent cloaca and anal atresia had multiple episodes of urinary tract infection and related end-stage renal disease. As renal replacement treatment, the patient had a history of hemodialysis through upper extremity TDC, and renal transplantation, which ended with chronic rejection. After the transplant rejection, the patient underwent hemodialysis through

femoral TDC because of chronic occlusion of the vena cava superior (VCS), which was associated with prior multiple central catheterization. The patient was referred to our interventional radiology department because of dysfunction of femoral TDC. An abdominal CT scan that was obtained because of catheter dysfunction showed acute occlusion of the infra-renal vena cava inferior (VCI) (Figure 1). On inspection, the tunnel was red and on palpation, there was tenderness over the tunnel. After releasing the cuff of the TDC, purulent discharge was observed. Hence, it was considered a tunnel infection. Because of the tunnel infection, the TDC was removed without the insertion of a new catheter on that side. To maintain hemodialysis, a non-tunneled dialysis catheter (NTDC) was inserted through the subclavian vein. The catheter tip was at the brachiocephalic vein as VCS was occluded. The patient underwent hemodialysis through NTDC and was under antibiotic treatment for the tunnel infection. After 20 days, the dysfunction of NTDC occurred. Despite the exchange of NTDC and insertion of a new one, effective hemodialysis through these NTDCs was not successful, mostly due to thrombosis. CT venography of central veins of the upper and lower extremities was ordered for the assessment of the current status of central veins. CT venography showed chronic occlusion of VSC, and subacute occlusion of iliac veins and VCI (Figure 2). An attempt of endovascular recanalization of VCS had failed. As the patient needed acute effective dialysis, insertion of femoral TDC through the occluded iliac vein and VCI was planned. Under sterile conditions and conscious sedation, the patent part of the common femoral vein was punctured, and a 4F vascular sheath was inserted. The DSA images obtained after contrast injection through the vascular sheath showed non-filling of iliac veins and VCI, and collateral vessels that eventually drain into the portal vein (Supplementary Figure 1). Occluded iliac veins were crossed with the guidewire loop technique. However, during the attempt of crossing occluded VCI, multiple times we ended up in intercostal or lumbar/ascending lumbar veins (Supplementary Figure 2). After multiple attempts with a 0.035-inch guide wire which was supported by a support catheter (NAVICROSS® Support Catheters, Terumo Europe), eventually, the occluded lumen of VCI was selected with the guide wire. Then, the support catheter was advanced over the wire to the right atrium (Supplementary Videos). After the balloon dilatation of occluded iliac veins and VCI, the 14.5F 44 cm TDC was advanced over the wire. The tip of TDC was in the right atrium (Figure 3). After the confirmation of good blood flow through TDC, the catheter's suture wings were fixed to the skin.

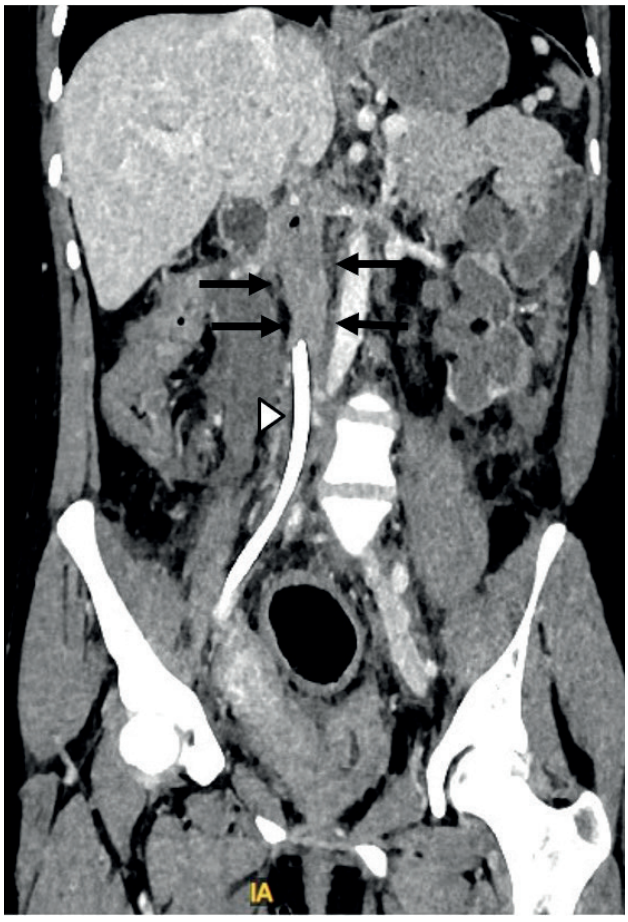

Figure 1. Abdominal CT coronal image shows dialysis catheter (arrowhead) and acute occlusion of infrarenal VCI (arrows).

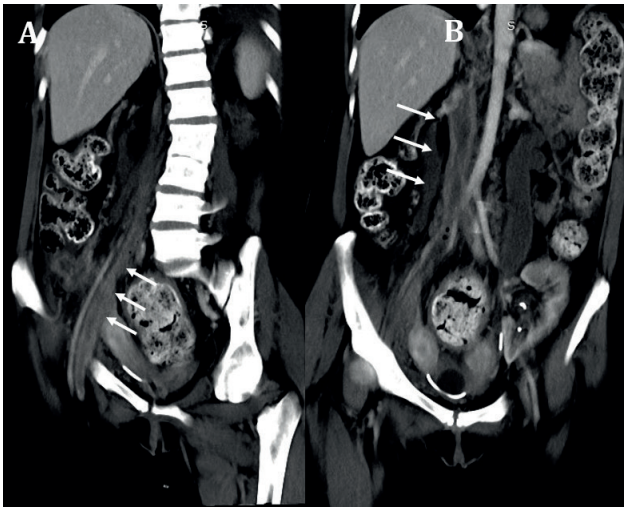

Figure 2. CT venography coronal images show subacute occlusion of iliac veins (arrows in A) and VCI (arrows in B).

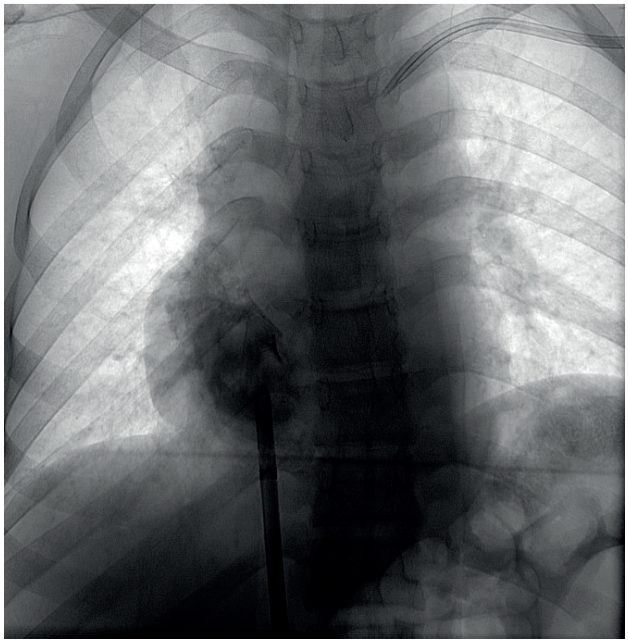

Figure 3. The DSA image shows the tip of the tunneled dialysis catheter in the right atrium, and left subclavian non-tunneled central catheter.

Left iliac veins were patent from the beginning, however, we did not use the left side because of the risk of vessel thrombosis and hampering future renal transplantation. The patient did not experience clinical signs of pulmonary thromboembolism during or after the femoral TDC insertion. Written informed patient consent was obtained before all procedures.

Maintaining venous access and catheter patency in patients undergoing hemodialysis through a central catheter is a dire necessity. In patients who depended on hemodialysis, when conventional venous accesses were run out, unconventional venous access techniques such as micropuncture through thrombus, trans-lumbar, transhepatic, trans-renal access, sharp recanalization, direct IVC cannulation, inside-out technique, and balloon or snare oriented puncture of non-patent central veins can be used to create venous access [3-7]. The decision of which unconventional venous access technique going to be used depends on the availability of requisite equipment and operator experience in that technique. In our case, there were two options, which were transhepatic access and TDC insertion through subacutely occluded lower extremity central veins, to consider. TDC insertion through occluded central veins was chosen instead of transhepatic access. This was because of our previous experience in this patient with NTDC insertions which were mostly complicated with vessel thrombosis. Hence, instead of puncturing the patent hepatic vein and taking the potential risk of patent vessel thrombosis and depleting the vein choice for future venous access. We chose not to puncture a patent vessel and insert the TDC through an already occluded vessel. Birgi et al., [1] published their experience with the insertion of TDC through acutely or subacutely occluded upper extremity central veins and they reported an overall 76% technical success rate. To the best of our knowledge, this is the first case report that reports the successful insertion of TDC through subacutely occluded lower extremity central veins.

CONCLUSION

In patients with subacute occlusion of the lower extremity central vein and exhausted upper extremity venous access, TDC insertion through an occluded central vein may be considered before proceeding with the puncture of patent unconventional veins such as hepatic veins for venous access.

Author contribution

Study conception: FÇ, FGE; Data collection: FÇ, OET; Analysis of data: OET, GH; Draft Manuscript preparation: FÇ, FGE, OET, GH. All authors approved the final version of the manuscript.

Ethical approval

Ethical approval was not required for this case report. This study has been anonymized to protect patient identity. Written informed consent was obtained from the patient for the procedure, and verbal consent was obtained for the publication of the case report.

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] Birgi E, Durmaz H. Placement of hemodialysis catheters with the help of the micropuncture technique in patients with central venous occlusion and limited access. Turk J Med Sci 2021;51(1):95-101. https://doi.org/10.3906/sag-2006-11

[2] Mandolfo S, Acconcia P, Bucci R, et al. Hemodialysis tunneled central venous catheters: Five-year outcome analysis. J Vasc Access 2014;15(6):461-5. https://doi.org/10.5301/jva.5000236

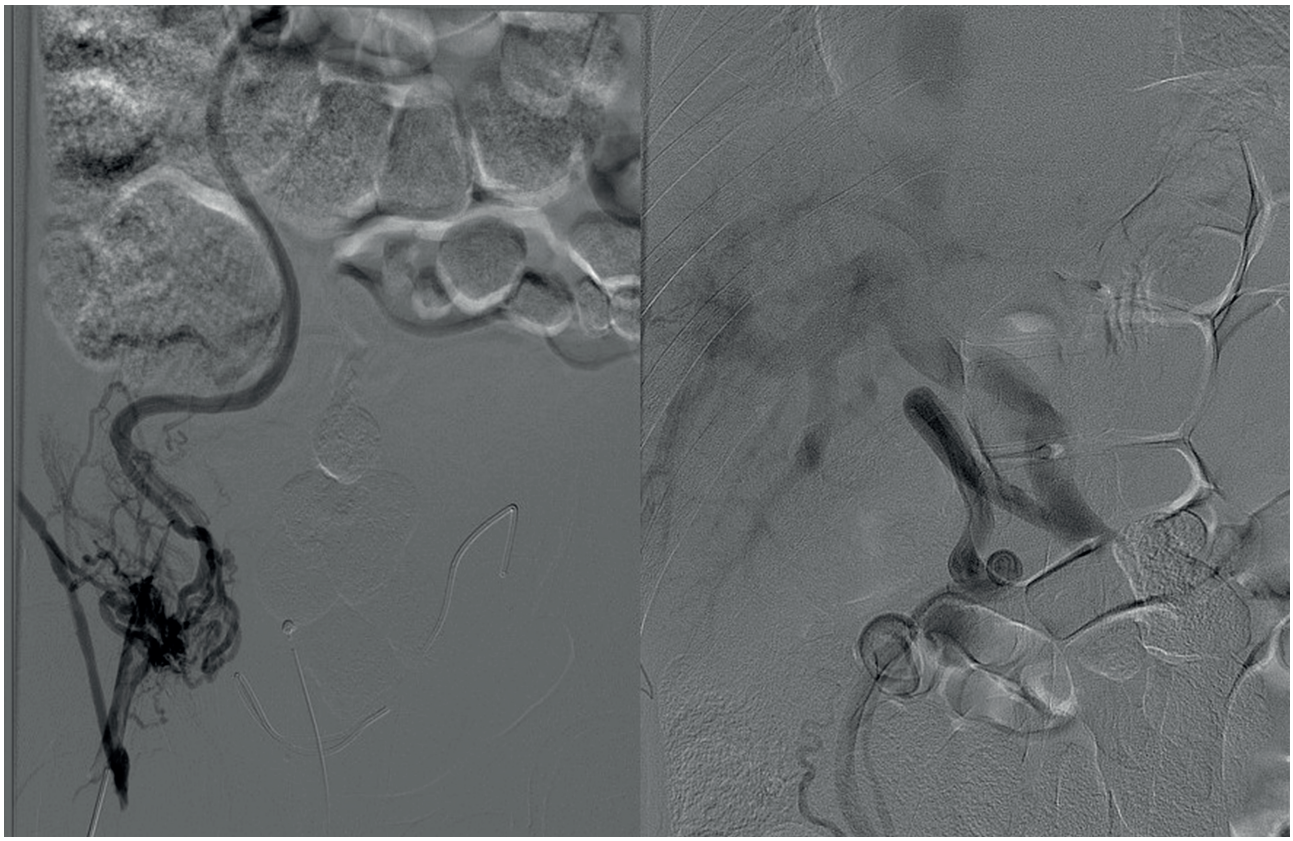
[3] Radhakrishnan Y, Dasari J, Anvari E, Vachharajani TJ. Tunneled femoral dialysis catheter: Practical pointers. J Vasc Access 2023;24(4):545-51. https://doi.org/10.1177/11297298211039633

[4] Lorenz JM. Unconventional venous access techniques. Semin Intervent Radiol 2006;23(3):279-86. https://doi.org/10.1055/s-2006-948767

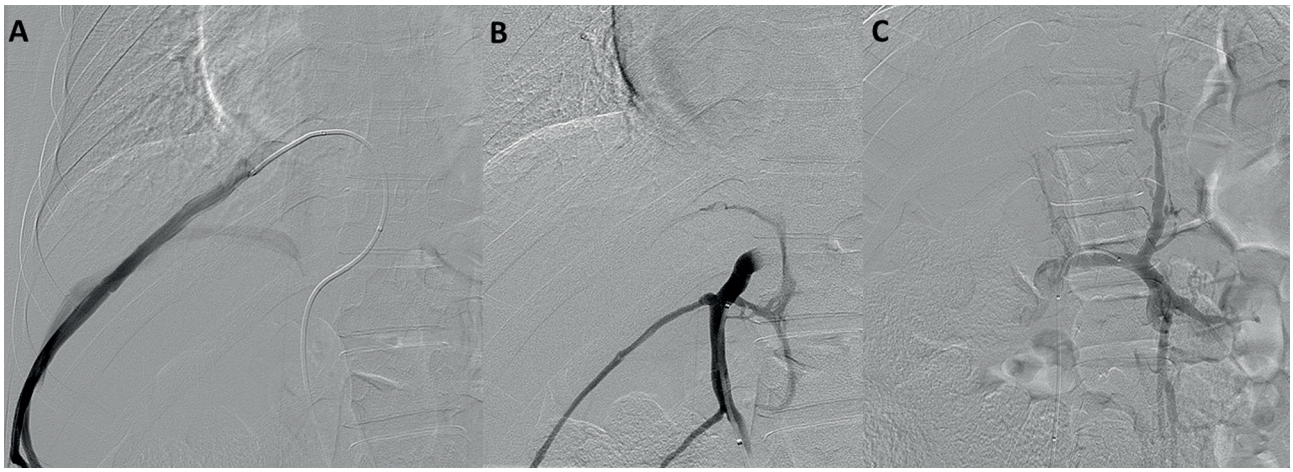
[5] Freeman BM, Tingen JS, Cull DL, Carsten CG. The inside-out technique for tunneled dialysis catheter placement with central venous occlusion. J Vasc Surg Cases Innov Tech 2019;5(3):350-5. https://doi.org/10.1016/j.jvscit.2019.03.015

[6] Hur J, Jeong B, Shin JH, et al. Transrenal Hemodialysis Catheter Insertion and Replacement in Patients with Upper Extremity Central Venous Access Exhaustion. Cardiovasc Intervent Radiol 2021;44(7):1121-6. https://doi.org/10.1007/s00270-021-02769-6

[7] Too CW, Sayani R, Lim EYT, Leong S, Gogna A, Teo TK. REcanalisation and Balloon-Oriented Puncture for Re-Insertion of Dialysis Catheter in Nonpatent Central Veins (REBORN). Cardiovasc Intervent Radiol 2016;39(8):1193-8. https://doi.org/10.1007/s00270-016-1383-5



Supplementary Figure 1. Images after contrast injection to the common femoral vein shows the collateral venous drainage to the portal vein.



Supplementary Figure 2. A: The catheter is in an intercostal vein. **B:** The catheter is in the ascending lumbar vein. **C:** The catheter is in the lumbar vein.