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Foreword

It is with great pleasure that we welcome you to the second issue of the Journal of European Internal Medicine Professionals (JEIMP) for 2025. As always, our mission is to create a space where diverse voices in medicine—especially from internal medicine and its many subspecialties—can come together to share knowledge, ask bold questions, and offer new answers. This issue brings together an exciting and thought-provoking set of articles that span from everyday clinical challenges to emerging frontiers in science. We begin with an exploration of Pain-related Factors in Hemodialysis Patients, shedding light on a common yet often underappreciated aspect of dialysis care. The next article, Mortality Rates and Predictors in Hospitalized COVID-19 Patients Receiving Hemodialysis for Different Conditions, takes us back to the height of the pandemic and helps us better understand the risks faced by vulnerable patient populations. On a more cellular level, the piece titled Progestin Repairs the Mitochondria Membrane Potential… offers fascinating insights into how hormonal therapy may influence mitochondrial function in reproductive health. In the field of neuro-oncology, Exploring the Role of Circulating Biomarkers in Glioblastoma Multiforme bridges the gap between research labs and clinical reality, aiming to bring hope and precision to the treatment of a devastating disease. Meanwhile, What's Missing in Diabetes Treatment? A Novel Agent Finerenone? challenges us to rethink the tools we use in managing diabetes and kidney disease. Finally, a timely Comment On: Pregnancy and The Kidneys invites reflection on a uniquely complex intersection of nephrology and obstetrics. Each of these articles tells a story—of curiosity, dedication, and a desire to improve patient care. We thank our authors, reviewers, and readers for being part of this ongoing journey, and we hope this issue will inspire new conversations and collaborations across the medical community.

Warmly,
The Editorial Board
Journal of European Internal Medicine Professionals (JEIMP)

Issue Content

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JEIMP
The Journal of European Internal Medicine Professionals

Original Article

Pain-related Factors in Hemodialysis Patients

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Abstract

Background: Pain is a prevalent issue among patients undergoing hemodialysis (HD). This study aimed to evaluate the prevalence of pain and identify factors associated with pain in HD patients.

Methods: Two hundred two HD patients participated in the study. Demographic and clinical data, pain characteristics, and sleep quality were recorded. Symptom burden and pain severity were assessed using the Edmonton Symptom Assessment Scale (ESAS) and the McGill-Melzack Pain (MGP) questionnaire.

Results: The majority of participants were male (59.9%), with a mean age of 59.6±12.7 years. Pain was reported by 80.2% of the patients and was significantly more prevalent among females (p=0.001) and individuals with lower educational levels (p=0.005). Median ESAS and MGP scores were 20 (range: 4-84) and 47 (range: 22-84), respectively. Patients reporting pain had significantly higher levels of CRP (p=0.044), parathyroid hormone (p=0.005), and higher ESAS scores (p=0.001). Sleep quality was impaired in 37% of patients. ESAS scores were significantly higher among females (p=0.003), those with impaired sleep quality (p<0.001), and regular analgesic users (p=0.002). MGP scores were significantly elevated in patients with diabetes (p=0.002), lower educational attainment (p=0.022), daily pain occurrence (p<0.001), and poor sleep quality (p<0.001). Additionally, patients with pain in multiple body regions reported higher MGP scores (p<0.001). There was a significant correlation between MGP scores, age (p=0.001), and ESAS scores (p<0.001).

Conclusion: Pain is highly prevalent among HD patients and is associated with female gender, lower educational level, elevated CRP, and higher parathyroid hormone levels. The severity of pain is particularly influenced by diabetes, low education level, and the number of painful body regions. Moreover, pain significantly impacts symptom burden and sleep quality.

Keywords: Hemodialysis, Pain, Quality of Life, Sleep

INTRODUCTION

Hemodialysis (HD) patients commonly experience various symptoms affecting multiple organs and systems, with pain being among the most frequent complaints (1). Although reported pain prevalence varies depending on the assessment methods used, it remains notably high (2,3). Pain severity in HD patients ranges broadly from mild discomfort to severe pain.

Pain significantly contributes to sleep disturbances and psychosocial challenges in HD patients (4). Additionally, it is closely linked with depression, decreased quality of life, increased disease burden, and impaired sleep quality (5). If left untreated, pain can result in shortened or missed dialysis sessions, increased hospitalization rates, and frequent visits to healthcare facilities (6). Furthermore, persistent pain has a detrimental impact on patient survival (7). Therefore, systematic assessment, effective management, and identification of factors related to pain are essential components of comprehensive care in HD patients.

This study aimed to evaluate the prevalence, intensity,

and factors associated with pain among HD patients.

METHODS

Patients

We conducted this study by face-to-face questionnaire at the hemodialysis unit of the Ondokuz Mayıs University. Inclusion criteria were ≥age 18 years, HD duration ≥one year, and adequate cognitive function. Exclusion criteria were the presence of cancer and/or overt infection. A total of 202 patients gave informed consent for participation.

Socio-demographic information such as age, gender, and educational status of the patients were questioned. Duration and etiology of chronic kidney disease (CKD), HD vintage, comorbidities, dialysis vascular accesses (fistula or catheter), and the polymerase chain reaction (PCR) positive COVID-19 history of the patients were recorded. The presence of pain was questioned in all patients. In patients with pain, the duration and frequency of pain, its effect on daily life and sleep quality, the use of drug therapy for pain, and whether they used alternative medicine for pain were determined. Hemoglobin, albumin, C-reactive protein (CRP), parathormone (PTH), urea reduction rate (URR), fractional urea clearance (KT/V), and other biochemical parameters were obtained from the medical records. Pain and symptom burden were assessed using the McGill-Melzack Pain (MGP) and Edmonton Symptom Assessment System (ESAS) questionnaires. The presence of pain for more than 3 months is characterized as 'chronic'.

McGill-Melzack Pain (MGP) Questionnaire

The McGill-Melzack Pain (MGP) questionnaire is widely utilized internationally to assess pain. The questionnaire comprises four sections that evaluate pain location, characteristics, temporal changes, factors influencing pain intensity, and the overall severity. Pain severity is determined by descriptive terms such as mild, uncomfortable, annoying, distressing, terrible, and unbearable. Additionally, pain intensity is quantified through a numerical scoring system ranging from 0 to 112 points. This questionnaire has been previously applied in assessing pain among hemodialysis patients (8). The Turkish version's validity and reliability were confirmed by Oksuz et al. in 2007 (9).

Revised Edmonton Symptom Assessment System

The Edmonton Symptom Assessment System (ESAS) assesses pain, fatigue, drowsiness, well-being, nausea, appetite, shortness of breath, depression, anxiety, and itching. Each symptom is rated on a scale of 0-10 (minimum-maximum).

STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS Statistics for Windows, version 15.0 (SPSS Inc., Chicago, IL, USA). Normality of data distribution was assessed with Kolmogorov-Smirnov and Shapiro-Wilk tests. Descriptive statistics were presented as means, medians, numbers, and percentages. The independent samples t-test was used to analyze numerical variables with normal distribution, while the Mann-Whitney U test was applied for variables without normal distribution. Chi-square analysis was conducted for comparisons of categorical data. Spearman's correlation test was employed to evaluate correlations. Statistical significance was set at a p-value of less than 0.05.

RESULTS

Most participants (59.9%) were male (mean age 59.6±12.7 years). Median HD duration was 3 years (1-22). The most common CKD etiologies were hypertension (43.1%) and diabetes (32.7%), respectively. AV fistula was used in 72.3%, and tunneled catheters were used in 25.7% as vascular access. 26.2% of the patients had COVID-19 infection (**Table 1**).

Most of the patients (80.2%) had pain. Almost all (90.1%) had chronic pain (≥12 months in 71% of the patients). The frequency of pain was 'daily' in 34% of the patients and 'a few days in a week' in 35.2%. When the patients were asked how often the pain affects their daily life, 43.8% answered 'sometimes', 29% 'often', and 8.6% 'always'. In addition, 37% of the patients stated that pain affected their sleep quality (**Table 2**).

Most of the patients (91.4%) used medications for pain. Frequently used medications are; paracetamol (56.8%), pregabalin/gabapentin (25.9%), and NSAID (21%). About one-fifth of the patients (16.7%) used medication regularly, and 54.9% used it only when needed. 26.5% of the patients used antidepressants, and 12.3% applied alternative medicine methods for pain. Apart from dialysis physicians, internal medicine physicians (34.6%), a nephrologist (17.9%), orthopedics (13.6%), and algology specialists (12.3%) were consulted for pain, respectively. Patients frequently had their pain medication prescribed by dialysis physicians (78.4%). Additionally, 25.7% and 44.6% of the patients were admitted to family and other specialist physicians, respectively (**Table 2**).

Analysis of ESAS and MGP Questionnaires

The patients' median ESAS and MGP scores were 20 (4-84) and 47 (22-84), respectively. According to the MGP scale, patients classified their pains as follows; 18.5% 'mild', 45.1% 'discomforting', 27.2% 'distressing', 8% 'horrible', and 1.2% 'excruciating'. The lower extremity (61.1%) was the most common site of pain. This was followed by the upper extremity (35.8%) and the lower back (13%). 29.6% of patients reported pain in at least two body regions. Pain frequency was higher in women (p=0.001) and lower educated (p=0.005). In patients with pain, CRP (p=0.044) and PTH (p=0.005) levels were higher. In addition, ESAS (p=0.001) scores were higher, too. However, age, CKD duration, CKD cause, dialysis

Table 1. Demographic, laboratory, and pain-related characteristics of patients

Parameters	Mean +/-SD
Hemoglobin (g/dL)	11.0 ± 1.3
Albumin (g/dL)	3.5 ± 0.3
Parathormone (pg/mL)	369 (14-2982)
URR (%)	71.8 (38.7-88)
Kt/V	1.5 (0.6-2.5)
(Ca) x (P)	43.4 (16-100)
CKD etiology, n (%)	
Hypertension	87 (43.1)
Diabetes mellitus	66 (32.7)
PKD	16 (7.9)
Glomerulonephritis	12 (5.9)
Others	12 (5.9)
Unknown	9 (4.5)
Vascular access, n (%)	
AV fistula	146 (72.3)
Tunneled catheter	52 (25.7)
COVID-19 history (positive)	53 (26.2)
Presence of pain, n (%)	162 (80.2)
Pain duration	
<3 months	16 (9.9)
3-6 months	13 (8.0)
7-12 months	18 (11.1)
>1 year	115 (71.0)
Pain frequency, n (%)	
Daily	55 (34.0)
Few days a week	57 (35.2)
Few days in a month	27 (16.7)
Rarely	23 (14.2)
Impact on quality of life, n (%)	
Never	2 (1.2)
Rarely	28 (17.3)
Sometimes	71 (43.8)
Most of the time	47 (29.0)
Anytime	14 (8.6)
Poor sleep quality,n (%)	60 (37)

*URR; urea reduction ratio, Kt/V; fractional urea clearance, (Ca)x(P); calcium phosphorus product, CKD; chronic kidney disease, PKD; polycystic kidney disease, AV; arteriovenous.

duration, comorbidity, vascular pathway, COVID-19 history, hemoglobin, albumin, URR, KT/V, and (Ca) x(P) product did not differ between groups (Table 3).

Females' ESAS scores were significantly higher than males. The MGP score was higher in those with lower education. Those with diabetes and coronary artery disease (CAD) had a higher MGP score than those without (p=0.003). No significant difference in ESAS score was observed according to comorbidities. ESAS scores were higher for limb pain (p=0,002) and back pain (p=0,003) and lower for head pain (p=0,002). MGP scores were higher for upper extremity (p=0.023) and back (p=0.003) pain and lower for headache (p=0.030). Those with two or more painful sites had a higher MGP score than those with pain in one site (p<0.001) (Table 4). ESAS and MGP scores were significantly higher in those whose pain interfered with daily activities and sleep (p<0.001). ESAS and MGP scores differed significantly according to pain frequency (p<0.001). ESAS and MGP scores were significantly higher in those with daily pain than in the others (Table 4).

Table 2. Characteristics of patients regarding pain management

Analgesic use, n (%)		148 (91.4)
Analgesic type,n (%)		
Paracetamol		92 (56.8)
Pregabalin/gabapentin		42 (25.9)
NSAID		34 (21.0)
Herbal supplement		31 (19.1)
Topical analgesic		18 (11.1)
Opioids		10 (6.2)
Others		7 (4.3)
Frequency of analgesic use, n (%)		
Regularly		27 (16.7)
Sometimes		18 (11.1)
When needed		89 (54.9)
Rarely		28 (17.3)
Antidepressant use,n (%)		53 (26.5)
Complementary or alternative medicine, n (%)		20 (12.3)
Specialties admitted for pain palliation other than dialysis physician	Internal medicine	56 (34.6)
	Nephrology	29 (17.9)
	Orthopedics	22 (13.6)
	Algology	20 (12.3)
	Neurology	11 (6.8)
	Neurosurgery	5 (3.1)
	Others	19 (11.7)
Prescribing pain medication	Dialysis physician	116 (78.4)
	Family physician	38 (25.7)
	Other physicians	66 (44.6)

Herbal supplement users had significantly higher MGP scores than others (p=0.045). ESAS and MGP scores were significantly different between groups according to frequency of analgesic use. The ESAS and MGP scores of patients who used analgesics regularly were higher than the others (p<0.05). The ESAS scores of patients who were prescribed analgesics by specialists were significantly lower than those who were not prescribed analgesics by specialists (p=0.043) (Table 5).

In the correlation analysis, the ESAS score was moderately correlated with the MGP score (r: 0.412; p<0.001). MGP score was weakly associated with age (r: 0.214; p=0.001).

DISCUSSION

Chronic kidney disease is a global problem with increasing prevalence, and patients' life is negatively impacted by the complications of CKD. Pain is a factor that has an impact on patients' quality of life and sleep. Most patients in our study had chronic and severe pain. Over 50% of HD patients experience pain (2,3). In this study, most of our HD patients (80.2%) experienced pain. On the other hand, about half of our patients had a level of pain that was severe or more severe. Pain severity affects quality of life, as is well known. Pain severity tends to be high in HD patients (2,3,10). The study by Er and colleagues showed that pain was intolerable in 6.7%, very severe in 10% and severe in

Table 3. Comparison of the patients according to the presence of pain

Factors	Pain (+) (n=162)	Pain (-) (n=40)	p-value
Age (years)	60.1 ± 12.6	57.7 ± 13.1	0.295
Gender (Female) (%)	74 (45.7)	7 (17.5)	0.001
Education level (Low)	104 (64.2)	16 (40)	0.005
CKD duration (years)	5 (1-40)	5 (1-23)	0.716
CKD etiology,n (%) • Hypertension • Diabetes	 68 (42) 57 (35.2)	 19 (47.5) 9 (22.5)	 0.527 0.126
Hemodialysis vintage	3.5 (1-22)	3 (1-20)	0.827
COVID-19 history	44 (27.2)	9 (22.5)	0.548
Hemoglobin (g/dl)	11.0 ± 1.3	10.8 ± 1.5	0.388
Albumin (g/dl)	3.5 ± 0.3	3.5 ± 0.2	0.516
CRP (mg/L)	9 (0.1-164)	4.6 (1-148)	0.044
PTH (pg/mL)	400 (14-2982)	279 (52-1324)	0.005
URR (%)	71.8 (38.7-88)	71.3 (48.6-85)	0.604
Kt/V	1.5 ± 0.3	1.5 ± 0.3	0.952
(Ca) x (P)	42.8 (16-100)	44.9 (20-98.4)	0.357
ESAS	21 (4-84)	13 (5-53)	0.001

CKD; chronic kidney disease, CRP; C-reactive protein, PTH; parathormone, URR; urea reduction ratio, Kt/V; fractional urea clearance, (Ca) x (P); calcium phosphorus product, ESAS; Edmonton Symptom Assessment Scale

31.7% of their patients (11). The frequency of pain is also a crucial issue. Er et al. also stated that 53.7% of their patients experienced pain at least once a week (11). Furthermore, almost all our patients had chronic pain, and a significant proportion (71%) had pain for over a year. Similarly, Gamondi et al. showed that the majority of HD patients (84%) experienced chronic pain (3).

It is a widespread pain that concerns the whole body in dialysis patients. Extremity pain was the most common in our patients. Similarly, Fleishman et al. show that foot pain was the most common site of pain in dialysis patients (62.5%) (12). Bone mineral disorders, osteoarthritis, and comorbid diseases such as diabetes could be responsible for this. In this study, patients with ≥ 1 painful region also had higher MGP scores. Similarly, severe pain was associated with ≥4 painful regions in the study by Fleishman et al. (12).

Approximately one-third of the patients stated that the pain affected their sleep. Also, pain severity is higher in those whose sleep quality is affected. Sleep quality is poorly affected in HD patients (11,13). Poor sleep quality is associated with depression (14). Similarly, increased pain severity causes sleep problems in HD patients, as shown by Harrison et al (15).

Pain frequency was higher in women. Samoudi and colleagues have shown that pain has a major effect on the quality of life of HD patients. Older patients, women and the uneducated are at high risk (16). In the study by Gamondi et al, similar to our findings, the female gender was the determining factor for the presence and intensity of pain in HD patients (3). There is a gender difference in pain sensitivity. Women report more considerable pains in more body areas than men. Some painful diseases are more common in women, and for many conditions, symptoms differ between women and men. Genetic, physiological, neuronal, hormonal, psychological, and social factors can mediate the difference in pain between men and women (17). Changes in estrogen plasma levels have been associated with recurrent pain in women (18). In addition, women seeking medical help more than men may cause a higher incidence of pain in women (17). Educational levels were lower among patients with pain in our study. Fleishman et al. reported a relationship between education level, income level, and pain in HD

Table 4. Comparison of patient and pain-related factors in terms of ESAS and MGP scores

Factors		ESAS Median (min-max)	P value	MGP Median (min-max)	P value
Gender	Female/Male	23(4-62)/17(4-84)	0.003	48.5 (22-84)/46(22-84)	0.302
Education level	Low/High	21 (4-84)/16.5(4-61)	0.057	52(22-84)/44(26-71)	0.022
Comorbidity	Diabetes(+)/(-)	20.5(4-84)/18.5(4-62)	0.210	52(24-84)/44 22-72)	0.003
	CAD(+)/(-)	21.5(4-84)/18.5(4-67)	0.291	53(22-84)/45.5(24-78)	0.003
COVID-19 history	(+)/(-)	16(4-50)/20(4-84)	0.236	53(26-77)/46(22-84)	0.316†
Pain frequency	Daily Few days a week Few days in a month Rarely	28 (11-84) 19 (4-61) 15 (7-51) 16 (4-45)	<0.001	55 (26-84) 51 (24-75) 45 (22-62) 41 (27-66)	<0.001
Pain impact on quality of life	Low/ High	17(4-52)/28(6-84)	<0.001	43(22-72)/56 (32-84)	<0.001
Impact on sleep quality	Yes/No	29 (4-84)/18(4-51)	<0.001	54.5(24-84)/ 44.5(22-75)	<0.001
Body pain region	Low extremity (+)/(-)	25(4-67)/16(4-84)	0.002	51(24-78)/45(22-84)	0.051
	Upper extremity (+)/(-)	21.5(4-67)/20.5(4-84)	0.987	54(27-78)/46(22-84)	0.023
	Head (+)/(-)	14(4-30)/22(4-84)	0.002	42(26-68)/49(22-84)	0.030
	Back (+)/(-)	28(13-67)/20(4-84)	0.00	60(37-75)/46(22-84)	0.003
The number of the painful region	One/Two or More	20(4-84)/25(4-67)	0.251	45(22-84)/57(34-78)	<0.001

ESAS; Edmonton Symptom Assessment Scale, MGP; McGill-Melzack Pain, CAD; coronary artery disease

Table 5. Comparison of patients' ESAS and MGP scores according to pain management-related characteristics

Factors		ESAS Median (min-max)	P value	MGP Median (min-max)	P value
Complementary or alternative medicine use	Yes/No	20 (4-43)/ 21.5 (4-84)	0.226	45 (32-78)/ 48 (22-84)	0.799
Analgesic use	Paracetamol (+)/(-)	20 (6-84)/ 22 (4-67)	0.997	48.5 (22-84)/ 46 (27-75)	0.855
	Pregabalin/gabapentin (+)/(-)	21.5 (4-67)/ 20.5 (4-84)	0.957	50 (26-78)/ 46 (22-84)	0.254
	NSAID (+)/(-)	21 (4-67)/ 20.5 (4-84)	0.608	45.5 (26-71)/ 48 (22-84)	0.386
	Herbal product (+)/(-)	19 (4-64)/ 21 (4-84)	0.198	42 (30-65)/ 51 (22-84)	0.045
Frequency of analgesic use	Regularly Sometimes When needed Rarely	35 (10-53) 19.5 (8-46) 19 (4-84) 21.5 (4-51)	0.002	62 (36-77) 44.5 (26-64) 46 (24-84) 42.5 (22-75)	<0.001
Antidepressant use	Yes/No	22 (4-61)/ 18 (4-84)	0.173	51 (26-77)/ 46 (22-84)	0.143
Physician prescribing analgesic	Dialysis physician (+)/(-)	20 (4-84)/ 22 (4-62)	0.495	48.5 (22-84)/ 46.5 (30-78)	0.742
	Other physicians (+)/(-)	17 (4-61)/ 22 (4-84)	0.043	47 (22-71)/ 51 (24-84)	0.640
	Family physician (+)/(-)	19.5 (4-62)/ 200.5 (4-84)	0.638	51 (22-78)/ 48 (26-84)	0.719

ESAS; Edmonton Symptom Assessment Scale, MGP; McGill-Melzack Pain, CAD; coronary artery disease

patients (12). The low level of education may make it difficult for patients to understand the causes of pain. In addition, these patients may have problems reaching the right resources for pain and using them appropriately.

CRP and PTH levels were higher in patients with pain. Inflammation can cause pain, and CRP levels are increased in various diseases that cause chronic pain (19-21). Secondary hyperparathyroidism can cause significant bone pain (22). A study showed that high PTH levels were a determinant of chronic pain (23). A positive relationship between pain and PTH levels in HD patients has been shown in another study (24). Similar to our study, Ghonemy and colleagues found a relationship between pain and elevated CRP and PTH levels. The authors have suggested that CRP is a sensitive marker for increased perception of pain (25).

Patients with pain are expected to have a high ESAS score. In our study, the ESAS score was elevated in patients with a high frequency of pain, those who stated that pain affected daily life and sleep quality. Since this scale assesses the burden of patients' symptoms such as fatigue, pain, nausea, anorexia, anxiety and depression together, scores may be higher in women. The frequency of depression and anxiety increases in kidney failure patients, associated with poor prognoses such as hospitalization and mortality (26). There is a bidirectional relationship between pain and depression. The patient's emotional state may change by pain. Depression also aggravates pain symptoms (27). In our study, MGP scores correlated with age. Although pain threshold and sensitivity change with age, the frequency of chronic pain increases with age. The prevalence of chronic pain in the general population over 65 is approximately 40% (28).

Pain symptoms such as joint pain, chest pain, headache and muscle pain are very common in people recovering from COVID-19 (29). Therefore, a higher frequency of pain can be expected in patients with COVID-19. However, our study did not detect any effect of COVID-19 status on patients' pain frequency, ESAS, and MGP scores. Since the frequency of chronic pain and the rate of analgesic use are high in our patients, evaluating the effect of COVID may require a more detailed examination. However, our study was not designed for this purpose.

Most of the patients in our study received pharmacological treatment for pain management. One in five used non-pharmacological treatment for pain. Paracetamol is the first choice of non-opioid drug in HD patients. NSAIDs can decrease residual renal function and cause gastrointestinal bleeding, uncontrolled hypertension, and hyperkalemia, but they can be used by closely monitoring the side effects (30). These concerns can explain the lower rates of NSAID use in our study. In the study of Fleishman et al., 66.1% of the patients used pharmacological pain treatment (12). However, analgesic treatment rates in patients were not expressed. In addition, in the same study, 24.5% of the patients used non-drug treatments for pain. Very few opioids have been prescribed to our patients. The prescribing policy in our country and the fear of the side effects may be responsible for this situation.

Non-dialysis CKD patients frequently prefer herbal products, but these treatments may increase the risk of kidney failure (31). On the other hand, there may be interactions between pharmacological agents and herbal supplements. Bhall et al. stated that kidney failure patients must inform their physicians before using herbal products, posing a significant health risk (32). By evaluating the pain characteristics and causes, as well as the treatments for pain palliation, patients can be prevented from being exposed to the side effects of these products.

In our study, we found that the majority of patients presented with pain complaints to physicians other than the dialysis physician and the nephrologist.

There are two important reasons for admitting to other specialties. First, as in previous studies, pain palliation cannot be adequately achieved in most dialysis patients, and patients seek different treatments (2). Secondly, because the causes of pain differ, patients apply to other specialties. These reveal the importance of a multidisciplinary approach to pain management.

Limitations of the Study

There are a number of limitations to our study. Our study was single-center and the number of patients was limited. Multicentric studies involving more patients will help overcome limitations in understanding and addressing pain-related problems. This survey study may not be sufficient to explain some cause-and-effect relationships. In addition, the pain etiology of the patients was not evaluated (neuropathic, ischemic, degenerative, etc.). Although the effect of pain on sleep and daily life has been questioned, clinical conditions that have been shown to affect pain, such as depression and anxiety levels, have not been studied. However, evaluating pain-related factors and symptom burdens in a large patient group makes our study powerful. On the other hand, in our study, the evaluation of both pain and pain-related quality of life markers and symptom burden with 2 different scales provided a more objective evaluation in patients.

CONCLUSION

Our findings underscore that pain is a prevalent and significant problem among HD patients. Regular assessment and monitoring of pain can enhance the quality of life for these individuals. Increased awareness and early detection of pain may facilitate timely interventions, ultimately improving patient outcomes and their overall dialysis experience.

DECLARATIONS

Ethics committee approval: Ondokuz Mayıs University Clinical Research Ethics Committee approved the study (OMU KAEK: 2021/412). The study was conducted in accordance with the principles of the Declaration of Helsinki for research involving human subjects.

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Original Article

Mortality Rates and Predictors in Hospitalized COVID-19 Patients Receiving Hemodialysis for Different Conditions

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Abstract

Background: To determine the mortality rates and predictors in patients hospitalized and treated for COVID-19 infection, who are also receiving hemodialysis (HD).

Method: This retrospective study included 104 patients who received HD and were hospitalized due to COVID-19 between March 2020 and 2021. Hospitalized patients who received HD were categorized into three groups: maintenance HD (MHD) patients, those receiving HD due to acute kidney injury (AKI) or chronic kidney disease (CKD), and those receiving HD due to AKI without CKD.

Results: Sixty-four (62%) of the patients were male. The mean age of the patients was 68±13 years. 37 were receiving MHD, 41 were receiving HD due to AKI on CKD, and 26 received HD due to AKI without CKD. 12(32%) of MHD patients and 29(71%) of patients receiving HD due to AKI on CKD died (p=0.002). Of the patients receiving HD due to AKI without CKD, 26(100%) died. Patients receiving HD due to AKI without CKD had the highest mortality rate compared to both MHD and AKI in CKD groups (p<0.001). Factors predicting mortality included lymphopenia, HD due to AKI on CKD, a more than two-fold increase in AST, the requirement of mechanical ventilation, and elevated d-dimer levels.

Conclusions: We showed high mortality in all patients receiving HD for different clinical conditions. These findings highlight the necessity of close monitoring and early intervention in COVID-19 patients who received HD.

Keywords: Hemodialysis, Chronic Kidney Disease, Mortality, Acute Kidney Injury, COVID-19

INTRODUCTION

Patients with chronic kidney disease (CKD) are at increased risk of Coronavirus Disease 2019 (COVID-19) infection and its complications due to neutrophil and monocyte dysfunction, impaired T-cell activation, and a diminished humoral response (1). Additionally, multiple factors—such as reduced clearance of inflammatory mediators, oxidative stress, frequent infections, metabolic acidosis, and technical aspects related to dialysis—contribute to a state of chronic inflammation in patients receiving maintenance hemodialysis (MHD). These factors further increase susceptibility to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection (2). Moreover, patients undergoing hemodialysis (HD) have a higher risk of COVID-19 transmission because they receive treatment in crowded and enclosed dialysis units three times per week (3). Therefore, the risk of COVID-19 infection and its complications is significantly elevated in patients with CKD. In addition, COVID-19 infection may present atypically in this patient population (4).

Patients undergoing MHD may require hospitalization due to COVID-19. Furthermore, patients with or without CKD who are hospitalized for COVID-19 may develop acute kidney injury (AKI). In such cases, renal replacement therapy (RRT) may be necessary. Previous studies have reported that 37% of patients hospitalized

with COVID-19 develop AKI, and 14% of these patients require RRT (5).

COVID-19 infection may lead to kidney injury ranging from subclinical AKI to AKI necessitating RRT (5,6). The primary mechanism is multi-organ dysfunction; however, electron microscopic examinations have demonstrated the presence of SARS-CoV-2 in renal tubules, suggesting that direct viral cytopathic effects may also contribute to AKI (7). It has been reported that COVID-19 patients who develop AKI have a significantly higher risk of in-hospital mortality (8). Additionally, a study conducted in Turkey found that mortality rates were higher among patients with CKD, AKI, and those undergoing HD who contracted COVID-19 compared to the general population (9).

This study aimed to determine the mortality rates and predictors in patients hospitalized due to COVID-19 who were receiving HD for different clinical indications.

METHODS

Study Protocol and Patients

This retrospective study was conducted at the Hemodialysis Unit of the Nephrology Clinic at Dışkapı Educational and Training Hospital. A total of 104 patients aged ≥18 years who were hospitalized for COVID-19 and received HD between March 2020 and March 2021 were included in the study. Patients were classified as having COVID-19 infection if they tested positive for COVID-19 by real-time polymerase chain reaction (RT-PCR) and had clinical findings consistent with the disease. Patients with thoracic computed tomography (CT) findings suggestive of COVID-19 but a negative RT-PCR test result were not included. Patients under 18 years of age, those who were transferred to another center during hospitalization, and those with insufficient follow-up data were excluded from the study. Additionally, patients referred from another hospital were excluded. The evaluated parameters were recorded retrospectively, and patients with missing data were not included in the analysis. The flowchart illustrating the selection of the study population is presented in **Figure 1.**

The following parameters were recorded during the first HD session following hospitalization: age, sex, COVID-19 severity, comorbidities, smoking history, and the use of renin-angiotensin-aldosterone system (RAAS) blockers. In addition, laboratory findings were documented, including serum creatinine, hemoglobin, leukocyte count, lymphocyte count, platelet count, D-dimer, C-reactive protein (CRP), albumin, aspartate aminotransferase (AST), ferritin, and lactate dehydrogenase (LDH). Radiological lung evaluations, COVID-19 treatments, intensive care unit (ICU) admissions, mechanical ventilation (MV) requirements, and mortality status were also recorded. Pulmonary findings associated with COVID-19 were assessed based on thoracic CT reports. Mild disease was defined as the presence of mild clinical symptoms without CT-confirmed lung involvement. Moderate disease was defined as CT-confirmed lung involvement accompanied by fever, cough, and dyspnea. Severe disease was diagnosed in patients presenting with at least one of the following criteria: respiratory rate ≥30 breaths per minute, oxygen saturation ≤93%, or an arterial partial pressure of oxygen (PaO_2)/fraction of inspired oxygen (FiO_2) ratio ≤300 mmHg. Additionally, patients exhibiting more than 50% progression in lung involvement within 24–48 hours on radiological imaging were classified as having severe disease. Critical illness was defined by the presence of shock, multiple organ failure, ICU admission, or respiratory failure requiring MV (12).

STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). The normality of data distribution was assessed using the Shapiro-Wilk test, histograms, and Q-Q plots. Parametric data are presented as mean ± standard deviation, while non-parametric data are presented as median (minimum–

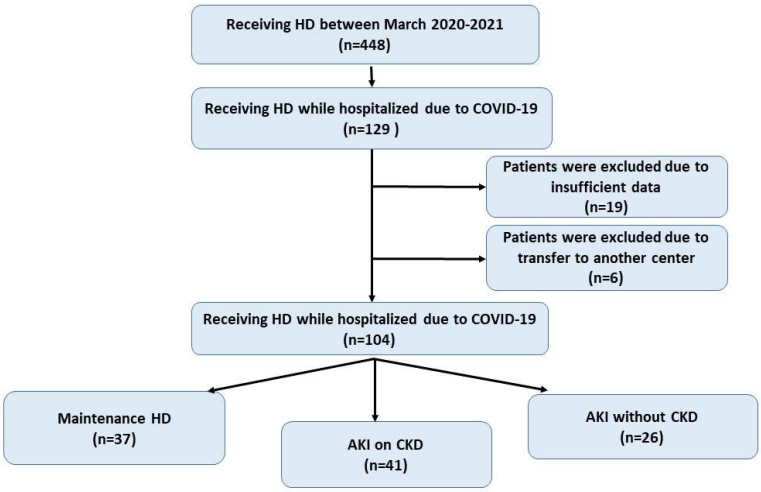

Figure 1. Flow chart illustrating the study population selection

Table 1. Demographic, clinical, and laboratory characteristics of patients

	All Patients (n=104)	Maintenance HD (n=37)	HD due to AKI on CKD (n=41)	HD due to AKI without CKD (n=26)	p-value
Gender, female, n (%)	40 (39)	18 (49)	17 (42)	5 (19)	0.054*
Age, mean ± SD	68 ± 13	61±14	71±11	71±13	0.001&
Comorbidities, n (%)					
HT	80 (77)	28 (76)	33 (81)	19 (73)	0.762*
DM	62 (60)	21 (57)	29 (71)	12 (46)	0.110*
CAD	40 (39)	15 (41)	18 (44)	7 (27)	0.555*
COPD	25 (24)	8 (22)	9 (22)	8 (31)	0.812*
Smoking, n (%)	44 (42)	10 (27)	15 (37)	19 (73)	0.001*
RAAS Blocker Use, n (%)	39 (38)	6 (16)	18 (44)	15 (58)	0.016*
Severe-Critical COVID-19, n (%)	64 (62)	13 (35)	29 (71)	22 (85)	<0.001*
Thorax CT Findings, n (%)					
Unilateral, single focus	13 (13)	9 (24)	3 (7)	1 (4)	0.025*
Unilateral, multiple foci	24 (23)	10 (27)	8 (20)	6 (23)	0.734*
Bilateral diffuse involvement	68 (65)	18 (49)	31 (76)	19 (73)	0.028*
Treatment, n (%)					
Favipiravir	99 (95)	34 (92)	39 (95)	26 (100)	0.334*
Glucocorticoid	72 (69)	16 (43)	33 (81)	23 (89)	<0.001*
Tocilizumab	31 (30)	6 (16)	13 (32)	12 (46)	0.036*
Convalescent Plasma	22 (21)	6 (16)	8 (20)	8 (31)	0.359*
Laboratory Findings (at HD initiation)					
Hemoglobin (g/dL), mean ± SD	9,8 ± 1,9	9,7 ± 1,7	9,9 ± 1,8	9,9 ± 2,2	0.938&
Leukocyte (mm^3), median (min-max)	9900(1200-54400)	6500 (2100-38000)	11400 (1400-49600)	19200 (1200-54400)	<0.001!
Lymphocyte (mm^3), median (min-max)	600 (200-5800)	700 (200-2500)	500 (200-1700)	600 (200-5800)	0.056!
Platelet (10^3/ mm^3), median (min-max)	170 (36-556)	181 (69-404)	157 (36-515)	165 (39-556)	0.958!
Creatinine (mg/dL), mean ± SD	5,7 ± 2,4	7,0 ± 2,6	5,3 ± 2,1	4,6 ± 1,5	<0.001&
AST (U/L), median (min-max)	32 (2-8110)	21 (8-504)	32 (2-2820)	114 (17-8110)	<0.001!
LDH (U/L), median (min-max)	468 (98-4687)	273 (98-1066)	562 (134-4464)	710 (237-4687)	<0.001!
Albumin (g/dL), mean ± SD	2,8 ± 0,5	3,1 ± 0,4	2,6 ± 0,4	2,5 ± 0,4	<0.001&
CRP (mg/L), median (min-max)	127 (1-529)	103 (1,4-439)	110 (8,1-529)	167 (1-437)	0.030!
Ferritin (µg/L), median (min-max)	1286 (70-59618)	1615 (263-12623)	1216 (70-51367)	1939 (176-59618)	0.186!
D-Dimer (mg/L), median (min-max)	3,0 (0,2-20,5)	2,3 (0,2-20,5)	3,4 (0,7-20,5)	3,8 (0,6-20,0)	0.020!
Evaluation of Laboratory Findings Based on Reference Ranges, n (%)					
Leukopenia, (<4000/mm^3)	4 (4)	2 (5)	2 (5)	0	0.306*
Lymphopenia, (<1500/mm^3)	70 (67)	21 (57)	30 (73)	19 (73)	0.142*
Thrombocytopenia, (<150x10/mm^3)	43 (41)	13 (35)	18 (44)	12 (46)	0.516*
CRP increase (>ten-fold)	85 (82)	28 (76)	33 (81)	24 (92)	0.195*
LDH increase (>two-fold)	54 (52)	3 (8)	29 (71)	22 (85)	<0.001*
AST increase (>two-fold)	27 (26)	2 (5)	10 (24)	15 (58)	0.045*
Clinical Outcomes					
Length of Hospital Stay, days, median (min-max)	9 (2-45)	8 (3-45)	8 (2-28)	12 (2-25)	0.355!
ICU admission, n (%)	64 (62)	13 (35)	27 (66)	24 (92)	<0.001*
Mechanical ventilation, n (%)	61 (59)	9 (24)	27 (66)	25 (96)	<0.001*
Mortality, n (%)	67 (64)	12 (32)	29 (71)	26 (100)	<0.001*

AKI, acute kidney injury; CKD, chronic kidney disease; HD, hemodialysis; ICU, intensive care unit; SD, standard deviation; CT, computed tomography; HT, hypertension; DM, diabetes mellitus; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; RAAS, renin angiotensin aldosterone system; CRP, C-reactive protein; AST, aspartate transaminase; LDH, lactate dehydrogenase. *Chi-square or Fisher's exact test. & One-way ANOVA test. ! Kruskal Wallis test

maximum). Categorical variables are presented as frequency (percentage). The Kruskal-Wallis test was used to compare non-parametric data among more than two groups, and the Mann-Whitney U test was used for pairwise comparisons. Parametric data between two independent groups were compared using Student's t-test, and data from more than two groups were compared using analysis of variance (ANOVA).

Table 2. The comparison results between surviving and deceased patients

Characteristic	Surviving Patients (n=37)	Deceased Patients (n=67)	p-value
Gender, female (n (%)	18 (49)	22 (33)	0,113*
Age, years, mean ± SD	61 ± 13	71 ± 12	<0,001&
Comorbidities, n (%)			
HT	31 (84)	49 (73)	0,217*
DM	24 (65)	38 (57)	0,417*
CAD	14 (38)	26 (39)	0,923*
COPD	8 (22)	17 (25)	0,668*
Smoking, n (%)	13 (35)	31 (46)	0,271*
RAAS blocker use, n (%)	9 (24)	30 (45)	0,039*
Mild-to-moderate COVID-19, n (%)	34 (92)	6 (9.0)	<0,001*
Laboratory findings at hemodialysis admission			
Hemoglobin (g/dL), mean ± SD	9,7 ± 1,6	9,9 ± 2,0	0,688&
Leukocyte (10^3/μL), median (min-max)	5,7 (2,1-25,4)	15,1 (1,2-54,4)	<0,001!
Lymphocyte (10^3/μL), median (min-max)	0,7 (0,3-2,5)	0,5 (0,2-5,8)	0,048!
Platelet (10^3/μL), median (min-max)	203 (78-515)	153 (36-556)	0,053!
AST (U/L), median (min-max)	22 (6-63)	62 (2-8110)	<0,001!
LDH (U/L), median (min-max)	269 (98-815)	593 (182-4687)	<0,001!
Albumin (g/dL), mean ± SD	3,1 ± 0,4	2,6 ± 0,5	<0,001&
CRP (mg/L), median (min-max)	78 (1-529)	161 (1-439)	<0,001!
Ferritin (μg/L), median (min-max)	836 (70-8372)	1672 (95-59618)	0,010!
D-Dimer (mg/L), median (min-max)	2,2 (0,2-20,5)	4,3 (0,6-20,5)	<0,001!
Evaluation of laboratory findings based on reference ranges, n (%)			
Leukopenia, (<4000/mm^3)	2 (5)	2 (3)	0,615*
Lymphopenia, (<1500/mm^3)	21 (57)	49 (73)	0,088*
Thrombocytopenia, (<150x10/mm3)	10 (27)	33 (49)	0,028*
CRP increase (≥ten-fold)	25 (68)	60 (90)	0,005*
LDH increase (≥two-fold)	4 (11)	50 (75)	<0,001*
AST increase (≥two-fold)	0	27 (40)	<0,001*
Clinical Outcomes			
Mechanical ventilation, n (%)	1 (3)	60 (90)	<0,001*
ICU admission, n (%)	6 (16)	58 (87)	<0,001*
Hospitalization duration (days), median (min-max)	8 (5-35)	9 (2-45)	0,624*

SD, standard deviation; HD, hemodialysis; DM, diabetes mellitus; HT, hypertension; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; RAAS, renin angiotensin aldosterone system; AST, aspartate transaminase; LDH, lactate dehydrogenase; CRP, C-reactive protein; CT, computed tomography. *Chi-square or Fisher's exact test. & Student's t test. ! Mann-Whitney U test

Categorical data were compared using either Fisher's exact test or Pearson's chi-squared test, as appropriate. Factors predicting mortality were evaluated using Cox regression analysis (Backward Likelihood Ratio method). Variables included in the mortality prediction model were age, lymphopenia, D-dimer level, HD due to AKI on CKD, a more than two-fold increase in AST, the requirement for MV, a more than two-fold increase in LDH, and albumin level. A p-value <0.05 was considered statistically significant.

Table 3. Predictors of mortality in patients hospitalized with COVID-19 and receiving HD

Variable	HR	95%CI for HR		p-value
		Lower	Upper	
Age	1.009	0.985	1.034	0.461
Lymphopenia	1.001	1.001	1.002	0.001
D-Dimer	1.066	1.014	1.121	0.012
HD due to AKI on CKD	1.816	1.058	3.116	0.030
AST increase (>two-fold)	2.005	1.123	3.580	0.019
Requirement of mechanical ventilation	4.433	1.703	11.544	0.002
LDH increase (>two-fold)	0.847	0.383	1.876	0.683
Albumin	0.998	0.613	1.626	0.993

RESULTS

A total of 104 patients were included in the study. Sixty-four patients (62%) were male. The mean age was 68±13 years. Among these patients, 37 were receiving MHD, 41 were HD due to AKI on CKD, and 26 were receiving HD due to AKI without CKD. The demographic, clinical, and laboratory parameters of the patients, as well as the comparison results among the three groups, are presented in **Table 1.**

Twenty-four MHD patients were receiving HD through an arteriovenous fistula. The median duration of HD in the MHD group was 8 years (range: 0–17 years).

Follow-up data demonstrated that the median duration of hospitalization was 9 days (range: 2–45 days). During follow-up, 64 patients (62%) required admission to the ICU, 61 patients (59%) required MV, and 67 patients (64%) died. The comparison results between surviving and deceased patients are presented in **Table 2.** Independent factors associated with mortality are shown in **Table 3.**

While 12 patients (32%) in the MHD group died, 29

patients (71%) in the AKI on CKD group died (p=0.002). All patients (n=26; 100%) receiving HD due to AKI without CKD died. The mortality rate was highest among patients receiving HD due to AKI without CKD when compared to both the MHD and AKI on CKD groups (p<0.001).

DISCUSSION

In our study, mortality was found to be high among patients receiving HD across three different clinical conditions. Furthermore, patients receiving HD due to acute kidney injury (AKI) without CKD had the highest mortality rates. In addition, the study identified several predictors of mortality in patients undergoing HD, including elevated AST, lymphopenia, increased D-dimer levels, HD due to AKI on CKD, and the requirement for MV.

In the general population, COVID-19-related mortality rates have been reported to range from 1.4% to 8% (13). In chronically immunosuppressed uremic patients, increased proinflammatory cytokine levels and decreased clearance of these cytokines contribute to higher mortality rates (14,15). In our study, the overall mortality rate was 64%. Specifically, mortality was 32% in patients receiving MHD, 71% in patients receiving HD due to AKI on CKD, and 100% in patients receiving HD due to AKI without CKD.

Previous studies involving MHD patients have reported similar mortality rates, with 24% in Italy, 31% in Spain, and 32% in New York (16–18). The mortality rates among MHD patients in our study were consistent with these findings. Similarly, a study conducted in Turkey demonstrated that 74% of patients with CKD who developed stage 3 AKI died, which aligns with our results (19). A large-scale study involving 5,449 patients found that 97% of the 285 patients with AKI requiring RRT also developed respiratory failure requiring MV (5). During the study period, 119 patients with AKI requiring RRT remained hospitalized, while 157 of the 166 patients (95%) died (5). The high mortality rates observed in our study among patients with AKI requiring RRT are consistent with the existing literature.

Additionally, AKI is directly associated with increased rates of ICU admission, MV requirement, and mortality, which may explain the high mortality rates observed in our cohort of patients with AKI (8). Mechanical ventilation has also been shown to be an independent predictor of AKI development and mortality in patients with AKI (20). Another study demonstrated that the need for MV is a significant risk factor for mortality in patients receiving HD due to AKI (21). Therefore, patients requiring both RRT and MV should be closely monitored, as they are at significantly increased risk of mortality.

The cytokine storm that occurs during COVID-19 infection can lead to fever and elevated ferritin levels, along with secondary hemophagocytic lymphohistiocytosis. It can also result in elevated AST and LDH levels due to hepatic involvement (22,23). A study conducted in the general population demonstrated an association between COVID-19-related mortality and lymphopenia (24). Similarly, lymphopenia was identified as a predictor of mortality in another study involving patients undergoing HD, consistent with findings in the general population (25. In addition, a separate study reported that a more than two-fold elevation in AST levels during hospitalization was associated with increased mortality in patients receiving MHD (26). It has also been shown that disease severity increases in COVID-19 patients who develop AKI as serum D-dimer levels rise (27). Another study investigating patients undergoing HD found that D-dimer levels were significantly higher in those who died from COVID-19 compared to those who survived (28). The same study also reported that the requirement for MV was a strong predictor of mortality in HD patients (28).

In our study, the predictors of mortality included lymphopenia, a more than two-fold increase in AST levels, elevated D-dimer levels, and the need for MV during follow-up. Although the mortality predictors identified in our study are consistent with previous reports in the literature, further comprehensive studies are needed to validate these findings.

Limitations of the Study

The limitations of our study include its retrospective design and relatively small sample size. Additionally, the absence of a control group and the single-center nature of the study further limit the generalizability of the findings. The fact that this was a single-center and retrospective analysis prevented the evaluation of potential variations in clinical practices among different HD centers. Another important limitation is that the study included only pre-vaccination data. COVID-19 vaccination has been shown to induce antibody responses and provide protection against infection in HD patients (29). Therefore, our findings may not be directly comparable to current patient outcomes in the post-vaccination era. Future multicenter, prospective studies that include vaccinated populations are necessary to validate and expand upon these results.

CONCLUSION

Our study demonstrated high mortality rates among patients receiving MHD, HD due to AKI on CKD, and HD due to AKI without CKD. In addition, we found that laboratory parameters such as elevated AST, lymphopenia, and increased D-dimer levels were independent predictors of mortality in patients undergoing HD. These findings highlight the critical

need for close monitoring and early intervention in COVID-19 patients requiring HD, particularly those with AKI. Future pandemic preparedness strategies should prioritize early identification and management of these high-risk populations. Moreover, prospective multicenter studies are needed to evaluate the impact of vaccination and post-COVID-19 complications in patients receiving HD.

DECLARATIONS

Ethics committee approval: This study was carried out according to the ethical rules and principles of the Declaration of Helsinki. Patient data was retrospectively accessed and anonymized before analysis. Approval for the study protocol was obtained from the Ethics Committee of Dışkapı Education and Training Hospital (approval date: 21 March 2022; approval number: 133/13).

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Original Article

Progestin Repairs the Mitochondria Membrane Potential by Preventing Membrane Hyperpolarization in Mitochondria Transferred Endometrial Stromal Cells

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Abstract

Background: The regenerative capacity of the endometrium is attributed to stem/progenitor cells. Despite their remarkable regenerative capacity, in some cases, impairments in regeneration can be observed. Endometrial mitochondria transplantation into the uterine cavity improves the uterine environment in Asherman's syndrome. Mitochondria transfer shows therapeutic advantages by supporting tissue metabolism and viability. However, the disruptive environment of the endometrium could affect mitochondrial health adversely. Increasing mitochondrial activity with progestin protects against apoptosis. The aim was to investigate the effect of progestin supplementation during exogenous mitochondrial transplantation. The study was designed as an in vitro cell culture of endometrial stromal cells. Mitochondria were isolated from the same cell line, representing autologous mitochondria transplantation.

Method: The transplantation of mitochondria was detected by fluorescence labeling of mitochondria. Viability was assessed by CCK8, and apoptosis was detected by AnnexinV/PI staining. Gene expression analysis was performed for Ki67, p38, and Erk1/2.

Results: Mitochondria were successfully transferred into endometrial stromal cells. The viability was not significantly altered due to the exogenous mitochondria, despite the increase in the reactive oxygen level. The addition of progestin is also well tolerated. The combined application of both mitochondria and progestin further supports the viability of cells without inducing the level of reactive oxygen species. Apoptotic levels were decreased in the presence of progestin even in the co-transfection group of mitochondria and progestin. The mitochondrial cell membrane was evaluated with JC-1 showing that the disrupted membrane potential was recovered by progestin, improving the damaged membrane potential of the mitochondria.

Conclusion: The damaged membrane potential improved in the presence of progestin, helping to improve the overall output of the mitochondrial transplantation.

Keywords: Dienogest, Stromal Cells, Membrane Potentials, Mitochondria, Progestin

INTRODUCTION

Endometrial tissue experiences cyclic changes during the menstrual cycle. Following the shedding during menstruation, the upper layer of the endometrium undergoes physiological alterations in response to estrogen and progesterone. Re-epithelialization of the endometrium is achieved by the formation of new luminal epithelial cells derived either from the activity of residual basalis glands or by mesenchymal-to-epithelial transition in the basalis. During this period, stromal/stem cells play significant roles in tissue regeneration (1). In menstrual/regeneration disorders, the recovery of endometrial tissue becomes impaired, as in Ascherman's syndrome or endometriosis, which leads to infertility. In these cases, the critically damaged endometrium could be repaired by intrauterine injected stem cells, which support the regeneration of epithelium and stroma by promoting cell proliferation (2,3). Several tissue sources of mesenchymal stem cells have been proposed in endometrial tissue repair of severe injury to the basal

layer of the endometrium (4-6). However, the ability of these stem cells to repair the damaged endometrium has some limitations (7). Drug therapy could support stem cell activities in several ways (8,9). As an alternative to drug or cell-based therapies, exosomes derived from cells have been shown to substantially improve tissue injury (10-12).

Mitochondria transfer between cells could support a diverse set of physiological processes by maintaining mitochondrial homeostasis (13). This cell-to-cell contact-mediated natural process was found to be common especially in some diseases, such as cardiovascular diseases and obesity (14,15). Mitochondria transfer occurs naturally, but this process can be induced by the activities of cancer cells (16,17). Contact-independent mitochondria delivery or transplantation into damaged tissue to maintain the tissue homeostasis is also proposed as an alternative for pharmaceutical applications. Mitochondria transplantation shows therapeutic advantages in human infertility and embryo quality (18). Hwang et al. indicate that mitochondria derived from endometrial organoids could play a crucial role in facilitating uterine repair, which in turn enhances fertility by restoring the disrupted metabolic conditions of the endometrium in Asherman's syndrome (19). There are several ways to transplant exogenous mitochondria into cells to prompt tissue regeneration. However, it is challenging to improve the transplantation medium to enhance the treatment efficiency in the delivery of mitochondria.

Mitochondria transplantation is still an experimental technique and information on mitochondria transplantation is quite limited, especially in endometriosis. To manage endometriosis symptoms, progestin treatment is a common and effective way. However, the effect of progestin on the transplanted mitochondria has not been revealed yet. In this study, it aim was to investigate the effect of progestin supplementation in the medium on exogenous mitochondria during transplantation. As the endometrial stromal cells play a central role in regeneration, the effect of mitochondria transplantation was analyzed in the presence of the progestin in vitro. The mitochondria derived from these cells were delivered into the cultured endometrial stromal cells.

METHODS

Isolation of Endometrium Stromal/Stem Cells (E-SCs)

E-SCs were isolated by an enzymatic method as previously described by Rencber et al. (20). Tissues were obtained from seven women without endometriosis (age, 42.0±1.29 years) who had operated for benign gynecological conditions. The female volunteers had regular menstrual cycles and had not received exogenous hormone treatment in the three months prior to surgery. The obtained E-SCs were cultured in DMEM/F12 medium supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C, under 5% CO2. Later, these cells were characterized by a flow cytometer with positive (CD44, CD73, CD90 and CD105) and negative markers (CD34 and CD45) for mesenchymal stem cells.

For the cell culture with progestin, Dienogest (Visanne, Bayer Weimer, Weimer, Germany) was used. It was dissolved in 20% DMSO in PBS at pH7.4. In drug supplementation assays, the final concentration of dienogest was adjusted to 10 nM to the level of EC50 dose to activate the progesterone receptor (9).

Mitochondria Isolation and Transplantation in Vitro

Mitochondria were isolated from E-SCs using Standard Hydrogen Electrode (SHE) buffer [0.25 M sucrose, 20 mM HEPES (pH 7.4), 2 mM EGTA, 10 mM KCl, 1.5 mM MgCl2 and 0.1% BSA]. Cells were suspended in SHE buffer at 4 °C, and cell membranes were disrupted by an injector using a 26G needle. The suspension was centrifugated at 250g for 5 min at 4 °C, the supernatant was transferred into another tube, and the mitochondria were recovered by centrifugation at 1500xg for 15 min at 4 °C. The pellet was dissolved in the SHE buffer. The Bicinchoninic acid method (SMART BCA kit, Intron) was used to determine the protein content in the mitochondria according to the manufacturer's instructions. Transfer of mitochondria is performed by adding mitochondria directly into the medium.

TMRM Staining and Detection of Exogenous Mitochondria in E-SCs

Before the isolation, mitochondria were stained first with Tetramethyl rhodamine methyl ester (TMRM), and then isolated from the cells. The aim of staining was to verify the transfer of exogenous mitochondria. During cell culture, cells were stained with TMRM dye at a final concentration of 10 µM for 20 min under standard culture conditions. The mitochondria were isolated and transferred as described. The mitochondria delivered by cells were fixed with PFA cells were visualized.

Cell Viability by CCK8

The viability of cells was determined by CCK8 assay (Elabscience Biotechnology, Wuhan,China). The CCK8 solution was diluted to 10% in RPMI1640 media without serum, according to manufacturer's instructions. Cells were incubated for 24 hours. Absorbance at 450nm was assessed using a microplate reader (VersaMax plus, Molecular Devices). The viable cell numbers were expressed as a percentage of absorbance observed in control cells to 100%.

Detection of Apoptosis and Level of Reactive Oxygen Species (ROS)

Apoptosis analysis was performed by Annexin V-FITC/PI Apoptosis kit (Elabscience), according to the kit instructions. Briefly, cells were washed with PBS, and

centrifuged at 250g, and cells were suspended in 500 µL of Annexin V Binding Buffer (5 µL of Annexin-V FITC and PI 5 µL) were added to each group. The cells were gently mixed and incubated at room temperature for 30 min. After incubation, the samples were analyzed using a FACS Calibur (BD Biosciences). The data obtained was later analyzed using the Cell Quest program (BD Biosciences).

To determine cellular ROS levels, 2′,7′-dichlorofluoresceindiacetate (DC-FDA) staining was performed. Briefly, the cells were washed twice with PBS and suspended in serum-free RPMI-1640 medium. DC-FDA dissolved in serum-free medium was added at a final concentration of 10 µM and incubated for 30 min at 37 °C, 5% CO2. At the end of incubation, the cells were washed again with PBS and analyzed by flow cytometry.

Mitochondria Membrane Potential

The mitochondria membrane potential was assessed by incubating cells in JC-1. The cells were incubated in the RPM 1640 culture medium supplemented with 0.5 mL tetrachloro-tetraethylbenzimidazol carbocyanine iodide 1 (JC-1) staining working solution and cultured for 20 min at 37°C. Cells were washed with PBS twice by centrifugating at 250g for 5 min. The membrane potential was detected by a flow cytometer. The cells were incubated in the RPM 1640 culture medium supplemented with 0.5 mL tetrachloro-tetraethylbenzimidazol carbocyanine iodide 1 (JC-1) staining working solution and cultured for 20 min at 37°C. Cells were washed with PBS twice by centrifugating at 250g for 5 min. The membrane potential was detected by a flow cytometer.

Gene Expression Analysis

After 24 hours following the transfer of mitochondria, total RNA was isolated by Aurum Total RNA Mini Kit (Bio-Rad, Hercules, CA, USA), and cDNA was synthesized by the cDNA synthesis kit (Thermo Scientific, Rockford, IL, USA), according to manufacturer's instructions. The amplification of target genes (Ki67, p38 and Erk1/2) was performed by Power SYBR-Green Master Mix (Thermo, Applied Biosystems Life Technologies, Carlsbad, CA, USA) with gene-specific primers in LightCycler 480-II (Roche) according to manufacturer's recommendations. Cp values were calculated by LightCycler 480 Software (release 1.5). Beta-actin was used as a housekeeping gene in normalization. ΔΔCp values were calculated with respect to control.

STATISTICAL ANALYSIS

The experimental groups were analyzed in triplicates (n=3). Statistical evaluation of the data obtained from the analyzes was performed using a parametric one-way ANOVA test for multiple group analyzes in the Minitap 14 program. Data acquired from the analyzes was analyzed using the student's paired t-test. Results were considered statistically significant at $p < .05$.

RESULTS

To assess the effect of progestin, E-SCs were culture in RPMI 1640 medium with progestin first for 24 hours. The mitochondria were transferred after then at the amount of 5 µg, equivalent to the protein concentration. The amount of mitochondria is almost equal to the mitochondria mass derived from the E-SCs at the number of 1x106 cells. As control, cells without treatment with progestin or mitochondria were used. The transfer efficiency was evaluated by flow cytometer, and it revealed that 90.17% ±1.22 of cells were positive after 24 hours of incubation, indicating that they were transplanted with exogenous mitochondria. However, the level of mitochondria penetrated inside the cells was not homogenous within the cell population (**Figure 1**). After 48 hours of incubation, the labeled mitochondria were still present in their cytoplasm with a signaling ratio of 84.61% ±5.24.

As the highest level of mitochondria was detected in the cells after 24 hours of transplantation, cells were analyzed at that time. The cell viabilities were not adversely affected by the transplanted mitochondria (**Figure 2A**). The progestin even affected viability. Remarkably, viability was improved in the cell

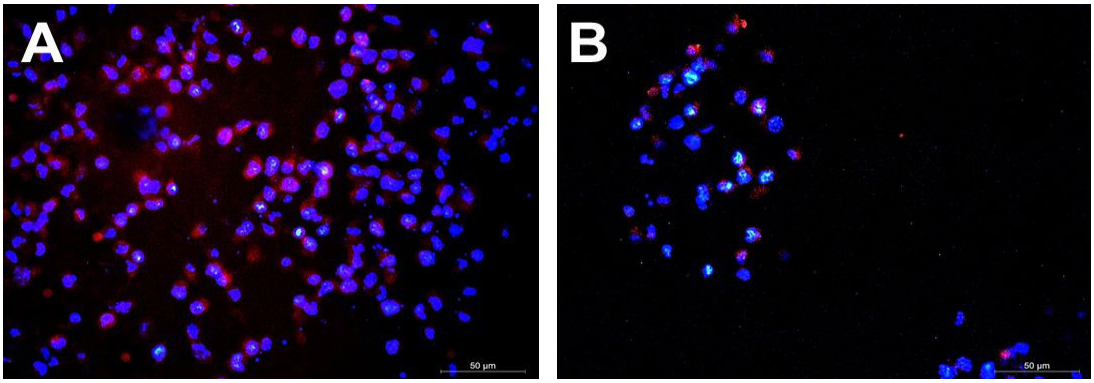

Figure 1. E-SCs after being transplanted with exogenous mitochondria derived from E-SCs, were labeled with TMRM dye and transplanted by adding the mitochondria to the culture medium. The cells were cultured and after 24 hours (A) and 48 hours (B), and mitochondria were visualized in red fluorescence. Cell nuclei was stained with DAPI, showing blue fluorescence. Scale bar,

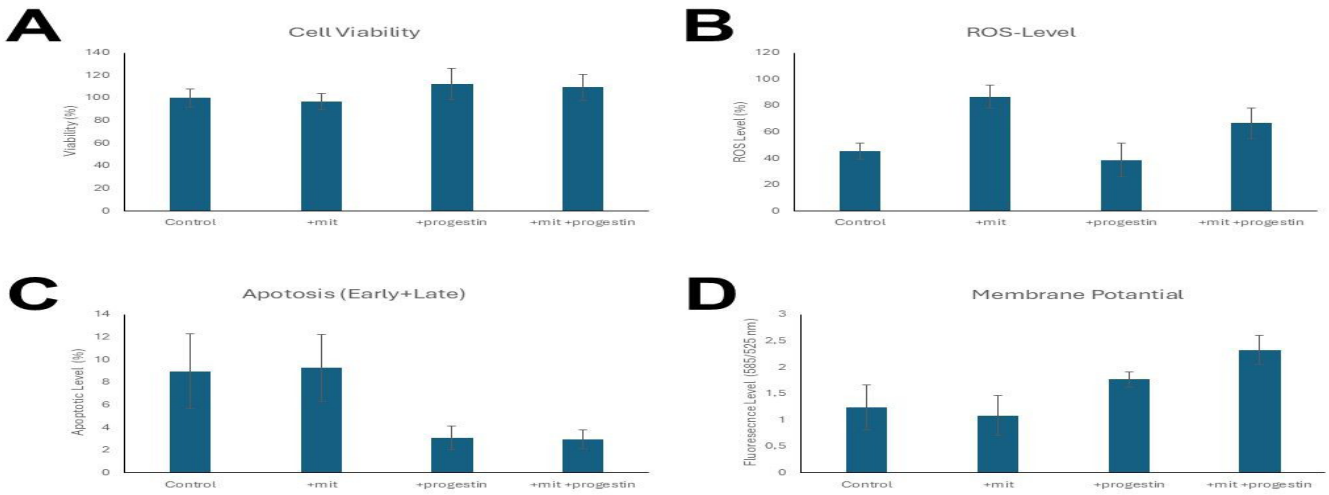

Figure 2. After 24 hours of transplantation, cells were analyzed for cell viability by CCK8, reactive oxygen species by DC-FDA staining, apoptosis by AnnexinV/PI staining, and mitochondrial membrane potential by JC-1 staining. The control group (without transplantation and progestin) was compared with sole mitochondria-transplanted group (+mit), sole progestin-treated group (+progestin), and the group (+mit +progestin) co-treated with mitochondria and progestin.

treated with both mitochondria and progestin. On the other hand, the ROS levels were found highest in the mitochondrial transplanted groups, indicating that the mitochondria might be damaged during isolation and the transplantation. The progestin negatively affected the production of the ROS level and decreased even below the level of the control group. This effect of progestin improved the mitochondria transplantation group in which the ROS level decreased in the co-treated cells (**Figure 2B**).

The apoptotic level of cells increased significantly after mitochondria transfer (**Figure 2C**). This increment was in parallel with the ROS level. Later, the increased apoptotic level was attenuated in the presence of the progestin, and this supportive effect of the progestin continued in the co-treated groups with mitochondria and progestin. This was also observed in the results of the mitochondrial membrane potential.

The attenuated level of ROS and apoptotic level due to the progestin could be linked with the activity of the mitochondria. The analyzes with JC-1 showed that the disruption in the mitochondrial membrane potential was improved due to the progestin, which improved the overall cell viability. The cell membrane potential level was found highest in the groups with progestin treated ones (**Figure 2D**).

The cellular response was evaluated also with the gene expression profile for Ki67, Erk1/2 and p38. Ki67 is a marker for cell proliferation. It was observed that either mitochondria transplantation or progestin treatment had an inductive effect on the proliferation (**Figure 3**). This could also explain the high level of cell viability even in the presence of an increased level of apoptosis and ROS levels. However, the treatment of both progestin and mitochondria did not show a combined improving effect so that the level of mitochondrial activity could be covered by the progestin effect in the cells. Although the reason was not clear, the expressions of Erk1/2 and

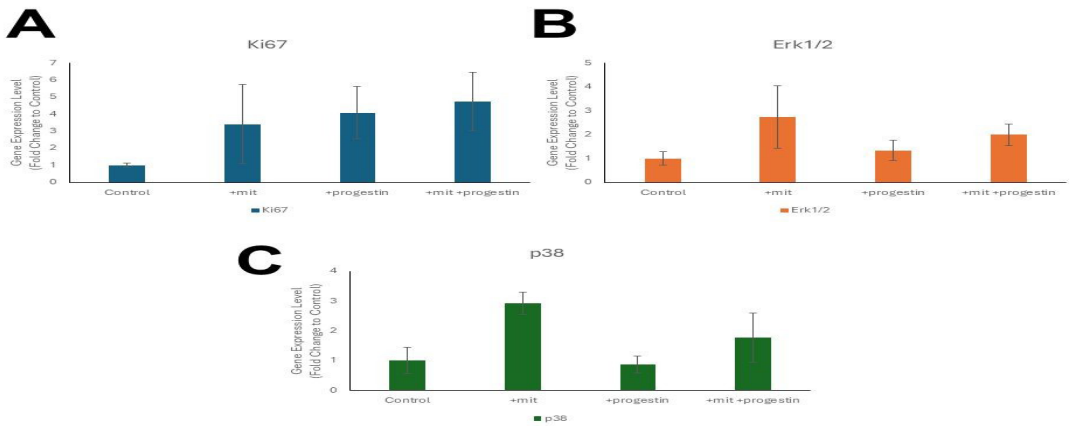

Figure 3. Gene expression analysis of mitochondria-transplanted and progestin-treated E-SCs for Ki67 (A), Erk1/2 (B), and p38 (C) after 24 hours of incubation following transplantation and/or progestin treatment. The control group (without transplantation and progestin) was compared with the sole mitochondria-transplanted group (+mit), the sole progestin-treated group (+progestin), and the group (+mit +progestin) co-treated with mitochondria and progestin. Gene expression levels were calculated as fold change relative to control cells.

p38, markers for the MAPK signaling pathway, were suppressed by the progestin. This suppression might improve the adverse effect of the exogenous mitochondria in the cells regulating the apoptotic levels by decreasing the apoptosis. Alternatively, the MAPK pathway might also induce mitophagy in the cells, which could explain the decreased level of transplanted mitochondria in cells after 48 hours compared to the cells after 24 hours.

DISCUSSION

Dienogest is a selective progestin that combines the pharmacological properties of 19-norprogestin and progesterone derivatives. Therefore, it has a strong progestational effect on the endometrium and a systemic anti-androgenic effect. There is a randomized placebo-controlled study showing that dienogest at a dose of 2 mg/day reduces pelvic pain and dysmenorrhea (21-23). In addition, case series have been published showing its effectiveness in both reducing lesion size and reducing pain in deep endometriosis and extrapelvic endometriosis (24). Progestins inoks prevent implantation of regurgitated endometrium, inhibit angiogenesis and the expression of matrix metalloproteinases, and reduce inflammation of endometriotic implants and the subsequent immune response (25,26). Dienogest (DNG) is a therapeutic drug used in the treatment of endometriosis. There is limited data regarding its mechanism of action on endometrial cells. Changes induced by DNG treatment in human endometrial stromal cells (E-SCs) were investigated using in vivo and in vitro models (27).

E-SCs are the cells at the center of endometrial regeneration. Any improvement in the viability of these cells would positively support the regenerative process of the tissues. So far, it is unknown how the cells are affected after mitochondrial transplantation in the presence of progestin. Progestin stimulates mitochondrial respiration by inducing biochemical and molecular parameters (28). The progesterone-induced increase in mitochondrial activity is not a precursor to apoptosis, but rather is protective (29). It was shown that the progestin positively affected the transplantation output, improving viability while decreasing the ROS and apoptotic level. Here, the mitochondrial membrane potential was focused on, as progestin improved the overall mitochondrial membrane potential. Depending on progestin-stimulated ATP production may contribute to prevention of cell apoptosis cooperatively with growth factor-stimulated signaling (30). The direct relation of progestin / progesterone has not been revealed, but the indirect relation was shown in studies in other cell lines (22). The energy status of the cell is key in the decision about cell proliferation. The higher the level of ATP present in the cells, the more it would improve the cellular metabolism in many aspects. This increased level of ATP could also support the antioxidant protective mechanism inside the cells, which provides defense against the increased ROS levels in the cells. Therefore, the induced ROS levels after the mitochondria transplantation did not undesirably affect viability and proliferation. Therefore, the progestin in the media improved the transplantation outcome. This challenges the dogma that exogenous mitochondria affect the cells toxically after transplantation due to increased ROS levels (16). The level of ROS still in cells is still a concern in viability. Its negative effect might be shed by the progestin.

The expression of Ki67, Erk1/2, and p38 genes constitutes a critical determinant of cellular viability and functionality (31). Ki67, a recognized marker of cellular proliferation, signifies the maintenance of functional integrity through the process of cellular division, a metabolic event necessitating substantial ATP. Under conditions of healthy mitochondrial transfer, these organelles effectively supply the requisite energetic substrates to support this process. Conversely, in instances of mitochondrial damage or dysfunction, characterized by disruption of the mitochondrial membrane potential, a reduction in ATP synthesis and an elevation in reactive oxygen species (ROS) generation are observed (32). This physiological stressor induces the transcriptional upregulation of Erk1/2 and p38, both members of the mitogen-activated protein kinase (MAPK) superfamily, which are integral to cellular stress response pathways (33). Notably, p38 has been implicated in the modulation of cellular proliferation under conditions of physiological stress. The addition of the progestin in the culture was known to support cell viability and proliferation. It was shown that progesterone can induce TERT expression in cells transiently within 12 hours after exposure. This induction was reported to mediate by MAPK signaling pathway (34,35). It was shown even to increase the telomerase activity by inducing TERT gene expression, which is expressed during the cell cycle process at the S-phase, where genomic DNA was duplicated. Addition of progestin positively affect the cell viability directly, but the mitochondria membrane potential was shown to improve in the presence of the progestin. Progesterone could have a wide range of beneficial effects on mitochondrial function. Interestingly, progesterone could protect neurons from injury by increasing the mitochondrial membrane potential and ATP production in neurons (36). In another study, progesterone was shown to improve mitochondrial function in sperm by increasing the mitochondrial membrane potential and ATP production (37). Progestin, like progesterone, could improve mitochondrial membrane potential and ATP production, while attenuating ROS production. In our study, it was shown that the progestin could also improve the cell viability and support the mitochondrial function in E-SCs.

Limitations of the Study

This study has several limitations. It was conducted entirely in vitro, which limits the ability to predict in vivo outcomes. The use of endometrial stromal cells from healthy individuals may not fully represent the cellular environment in disease states such as endometriosis or Asherman's syndrome. Additionally, the short observation period did not allow for evaluation of long-term effects or stability of the transplanted mitochondria. The molecular mechanisms underlying the effects of progestin on mitochondrial function were not fully explored. Further in vivo studies are necessary to confirm these findings and assess their clinical relevance.

CONCLUSION

Progestin could be used as an effective agent to control cell viability after mitochondria transplantation. In the treatment of endometriosis, progestin is commonly used. Therefore, tissue regeneration might be improved by mitochondria transplantation in addition to progestin treatment. Here, it was shown that the progestin did not disrupt the cellular event. Conversely, it supported the proliferation by decreasing the apoptotic levels. Mitochondria transplantation could possess some negative sides, as the process might, induce the ROS level in the tissue, even undesirably contributing to the degenerative nature of the endometrium. Progestin might be used to support mitochondria transplantation success in other cells or tissues. These findings also need to be confirmed through in vivo studies. In conclusion, cotreatment of mitochondria transfer and progestin has a significant effect on cell viability.

DECLARATIONS

Ethics committee approval: This study was carried out in accordance with the ethical principles of the Declaration of Helsinki. Patient data and tissue samples were used under ethical guidelines. Approval for the study protocol was obtained from the Ethics Committee of Kocaeli University (approval date: 12 March 2020; approval number: KU-GOKAEK 2020/55).
Informed consent form: Since the study was performed using previously collected tissue samples and conducted in vitro, and patient data were anonymized before analysis, an informed consent form was not required.
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The author has read and approved the final version of the manuscript.
Conflict of interest: The author declares that no conflicts of interest.
AI: Artificial intelligence tools were used to assist in language editing and improving the clarity of the manuscript. However, all scientific content, data analysis, and interpretations were carried out by the author, who bear full responsibility for the content and conclusions of this work.

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Review

Exploring The Role of Circulating Biomarkers in Glioblastoma Multiforme: Bridging The Gap Between Laboratory and Clinic

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Abstract

Glioblastoma multiforme is the most prevalent and aggressive primary malignant brain tumor in adults, characterized by significant intratumoral heterogeneity and resistance to conventional therapies. Despite improvements in surgical resection, radiotherapy, and chemotherapy with temozolomide, GBM remains incurable, with a median survival of 10–15 months. Current diagnostic modalities include magnetic resonance imaging and tissue biopsies, face early detection, real-time monitoring, and comprehensive tumor profiling limitations. These challenges underscore the urgent need for minimally invasive, highly specific, and sensitive diagnostic tools. Liquid biopsy has emerged as a promising alternative, enabling the detection of circulating biomarkers, including circulating tumor cells, cell-free nucleic acids, extracellular vesicles, and proteins from biofluids such as blood and cerebrospinal fluid. These biomarkers offer insights into tumor heterogeneity, therapeutic resistance, and progression while facilitating dynamic treatment response monitoring. This review explores the potential of circulating biomarkers in revolutionizing GBM diagnosis and management, focusing on their molecular characteristics, clinical utility, and limitations. By integrating these innovative approaches into clinical practice, liquid biopsy has the potential to significantly improve patient outcomes, heralding a new era in the diagnosis, prognosis, and therapeutic monitoring of GBM.

Keywords: Glioblastoma Multiforme, Circulating Biomarkers, Liquid Biopsy

INTRODUCTION

Glioblastoma multiforme (GBM) stands as the most common primary malignant brain tumor observed in adults, characterized by the highest mortality rate and notable aggressiveness. These tumors, known for their high mitotic activity and susceptibility to necrosis (1), account for approximately 14.5% of all Central Nervous System (CNS) tumors and nearly half of (48.6%) all malignant CNS tumors (2). Commonly located in the supratentorial region of the brain, GBMs are less frequently observed in the cerebellum, brainstem, and ventricles (3). GBMs arise as a result of uncontrolled growth of cells known as glia/neuroglia, which participate in neuronal activity, protect and support neurons, and cause symptoms such as headache, weakness, memory problems, personality changes, vision and speech difficulties, seizures, and paralysis arise due to the tumors compression of neighboring cells (1, 4). The majority of GBM patients, approximately 70%, face a poor prognosis, with a median survival ranging from 10 to 15 months and a 5-year survival rate of roughly 7% (5).

The etiology of GBM results from complex interactions between environmental factors and genetic predispositions, but the exact mechanisms of this relationship remain unclear (6). The carcinogenetic causes and mechanisms underlying the disease are not fully known. Although exposure to ionizing radiation is considered a risk factor for GBM, no definitive association has been found between GBM and environmental factors like tobacco use, electromagnetic fields, head injuries, and exposure to pesticides (4).

The conventional treatment approach for GBM currently involves surgical tumor resection, radiotherapy (RT), and chemotherapy (ChT) with the drug temozolomide

(TMZ) (7). The first step in treatment is to surgically remove as much of the tumor as possible, as this method is linked to longer progression-free and overall survival (OS) (8). Due to the diffuse infiltrative nature of GBMs, surgical resection is typically not curative. Instead, it aims to enhance survival, alleviate neurological symptoms, and improve patients' capacity to undergo postoperative treatments. The main features that complicate the treatment of GBM are the tumor's genetic, epigenetic, morphological, and histopathological heterogeneity. This type of heterogeneity encourages relapses that are resistant to treatment over time by causing the tumor to adapt to treatment and become resistant (9). Since resection is not a definitive therapeutic approach, RT and ChT are additionally administered to patients. While the OS in patients receiving RT alone is 12.1 months and the two-year survival rate is 10.4%, the OS is extended to 14.6 months, and the two-year survival rate is increased up to 26.5% with TMZ treatment in addition to RT (10). Despite this increase in survival with concurrent treatment with RT and TMZ, tumor progression and recurrence are typically inevitable due to the development of resistance to TMZ and hemotologic toxic effects (8). The challenges in treating GBM persist due to incomplete tumor resection, significant intratumoral heterogeneity, difficulty crossing the blood-brain barrier (BBB), and the immunosuppressive tumor microenvironment. The infiltrative nature of GBM makes it nearly impossible to achieve complete cellular-level resection. Additionally, hypoxic regions within the tumor create perivascular niches that support Glioblastoma-initiating cells (GICs). These self-renewing GICs contribute to the formation of more aggressive recurrent tumors that are resistant to both RT and ChT (11). Despite all these treatment options, GBM is still not completely curable, and recurrence rates after treatment are pretty high (2). Therefore, early diagnosis of these malignant tumors is crucial.

Considering today's technological advances, no screening tool or test has been developed to identify GBM before clinical symptoms emerge (2). GBM is diagnosed by computed tomography (CT) or magnetic resonance imaging (MRI) followed by biopsy for confirmation. However, the most important method for radiologically diagnosing GBM is MRI, as it outperforms CT with superior anatomical resolution, offers better delineation of GBM characteristics, and the ability to perform more advanced analyses such as brain tumor spectroscopy (5). There are also many limitations to the use of MRI for diagnosis. Due to the resolution limit of MRI, lesions as small as 2-3mm in size, particularly in the early stages, may be overlooked, which affects diagnostic accuracy and makes it difficult to detect early-stage tumors. Furthermore, it is not always possible to clearly distinguish GBM from other tumors and CNS diseases (12). GBM may therefore be mistaken for other brain tumors such as low-grade gliomas, brain metastases, or primary CNS lymphoma, as well as with nonneoplastic disorders like brain abscesses, demyelinating diseases, and hemorrhagic transformation of ischemic strokes (5). In some cases, MRI scans are also used to obtain prognostic information after treatment. In this case, lesions caused by tumor progression cannot be reliably distinguished from pseudoprogression (PsP). These treatment-related changes mimic tumor progression and could resolve independently over time. PsP occurs in 10-30% of GBM patients undergoing initial MRI scanning, typically within the first 12 weeks of treatment (13). In cases where surgery is not deemed appropriate, tissue analysis through stereotactic biopsies can help differentiate PsP from true tumor recurrence, but this further exposes patients to nonnegligible surgical risks.

Intraoperatively resected tumor tissue is needed for definitive diagnosis by histopathological examination. When tumor resection is not possible, or metastatic GBM is suspected, a fine needle aspiration biopsy is carried out from accessible areas (1). The intratumoral heterogeneity of GBM is an important factor in treatment compliance and resistance to treatment. In addition to the difficulty of obtaining tissue biopsy (TB) specimens and the serious potential complications of the procedures, the collected specimens may not fully represent the entire tumor due to intratumoral heterogeneity. Therefore, due to their invasive nature and limited sampling capacity, whole TBs cannot assess tumor activity in real-time (5). The risks involved in GBM TBs often prevent repeat sampling during tumor progression, restricting opportunities to monitor treatment response and identify therapeutic resistance at an early stage. The challenges posed by MRI and TBs emphasize the urgent and unmet clinical need for innovative, alternative, and complementary diagnostic techniques to improve the diagnosis, treatment, and follow-up of GBM patients (5). These challenges have led to the search for less invasive and more accurate methods in the diagnosis and treatment process. In this context, against the limitations of conventional diagnostic methods, circulating biomarkers have great potential for detecting and monitoring tumors in their early stages. The use of circulating tumor cells (CTCs), circulating cell-free nucleic acids (ccfNAs), extracellular vesicles (EVs), and circulating proteins (CP) are increasingly being investigated for monitoring patients' response to treatment with less invasive methods (14).

Biomarkers serve a vital role in the molecular profiling of the tumor, enabling more accurate prognostic predictions for personalized treatment strategies and early detection of treatment-resistant relapses. The aim of this review is to examine the potential of circulating biomarkers for diagnosing and monitoring GBM, to discuss new approaches that may provide alternatives to current methods, and to provide a perspective on how

these biomarkers can be used in clinical practice.

METHODS

The literature search was conducted in scientific databases including PubMed, Web of Science, and Scopus using keywords such as "glioblastoma multiforme", "circulating biomarkers", "liquid biopsy", "circulating tumor cells (CTCs)", "cell-free nucleic acids (cfNAs)", "extracellular vesicles (EVs)" and "circulating proteins (CPs)" to ensure a comprehensive and systematic review. We included research articles published in leading high-impact, peer-reviewed journals with strong experimental designs, in vitro and in vivo findings supported by clinical outcomes, and review articles published in the last 5 years discussing the advantages of liquid biopsy and circulating biomarkers and advances in their role in GBM. To ensure a balanced representation of the latest scientific advances, studies with large sample sizes, robust statistical analyses, and direct relevance to circulating biomarkers in GBM were prioritized for their potential importance. On the other hand, we excluded non-English language articles, conference abstracts, and articles that focused solely on preclinical models without human data.

LIQUID BIOPSY and CIRCULATING BIOMARKERS: PIONEERING A NEW ERA in DIAGNOSTICS and MONITORING of GBM

Tumor development is an extremely comprehensive and complex process, limited to physiological and metabolic changes and leading to various molecular and biochemical alterations (6). In order to manage this process effectively, the discovery of potential biomarkers is of great importance. Biomarkers are biological indicators that allow us to assess normal biological functions, pathological states, and pharmacological responses to therapeutic interventions with high sensitivity and specificity (15). In recent years, several diagnostic and prognostic biomarkers of aggressive glioblastomas have been identified and have contributed significantly to the accuracy of diagnosis and efficacy of treatment (6,16). Especially in GBMs, liquid biopsy (LB) is pioneering a new era (17), facilitating early diagnosis of GBM by recognizing neoplastic transformations, managing tumor progression, and optimizing patient follow-up by monitoring response to treatment (18). LB, as opposed to TB, is a minimally invasive diagnostic method with advantages, such as easy applicability, speed, cost-effectiveness, reproducibility, high sensitivity, real-time monitoring, and capacity to represent tumor heterogeneity (19,20). It provides detailed information about tumor evolution over time by detecting and quantifying tumoral contents released by tumors into biological fluids such as blood, cerebrospinal fluid (CSF), saliva, urine, and cyst fluid (17,19).

Different biological fluids offer distinct advantages and limitations in detecting circulating biomarkers for GBM, influencing their diagnostic accuracy and clinical applicability. Peripheral blood (PB) is the most investigated biological material for the determination of circulating biomarkers in GBM due to its advantages in accessibility, ease of collection, minimal invasiveness, and dynamically reflecting tumor progression. However, its diagnostic utility for GBM is limited by the selective permeability of the BBB, which restricts the release of tumor-derived molecules into circulation. As a result, blood-based biomarkers often exhibit lower sensitivity and specificity compared to other biological fluids, particularly in early-stage disease detection. In addition, CSF is also used as an ideal source of LB due to its potential for direct contact with the central nervous system and tumor microenvironment, making it a highly enriched source of GBM-related biomarkers (17). Biomarkers such as ccfDNA, EVs, and CPs are more abundant in CSF than PB, which is thought to lead to improved biomarker detection rates. However, CSF collection via lumbar puncture or ventricular catheterization is invasive limiting its routine use in clinical practice. Moreover, CSF sampling is not always feasible, particularly in patients with increased intracranial pressure or where tumor location restricts safe access. Urine, a completely non-invasive biological fluid, has recently emerged as a potential alternative for biomarker detection. EVs and microRNAs can be detected in urine samples of GBM patients. However, due to the low concentration of brain-derived molecules in urine, its diagnostic utility remains highly uncertain. Additionally, renal clearance and metabolic degradation further complicate biomarker stability, making urine-based GBM diagnostics less reliable compared to PB and CSF. Saliva has also been investigated as a potential biological fluid for detecting brain tumor biomarkers. Saliva collection is non-invasive and offers advantages such as ease of repeated sampling. EVs and ccfRNAs can also be detected in saliva, potentially reflecting tumor biology. However, the reliability of saliva-based biomarker detection for GBM remains highly questionable, as saliva primarily contains molecules derived from local oral and salivary gland tissues. Additionally, the low concentration of GBM-related biomarkers and potential contamination from other systemic factors limit its applicability in routine clinical settings. Cyst fluid collected from cystic GBM lesions presents a unique biological fluid with potentially high concentrations of tumor-derived biomarkers. Cystic components of GBM contain ccfDNA, EVs, and CPs, which may offer insights into tumor heterogeneity and progression. Cyst fluid may provide a more direct representation of tumor biology compared to PB. However, the clinical application of cyst fluid as a biomarker source is limited by the infrequent occurrence of cystic GBMs and the invasive

nature of fluid collection, which typically requires stereotactic aspiration. Given these differences, CSF remains the most promising biological fluid for GBM LB, while plasma offers a more practical alternative for longitudinal monitoring. However, due to their non-invasiveness, urine, and saliva-based biomarkers require further validation before they can be integrated into routine clinical practice. Despite its high biomarker content, cyst fluid only applies to a subset of GBM cases. Future research should focus on standardizing biological fluids selection criteria and optimizing detection methodologies to enhance the clinical applicability of LB in GBM.

Two types of biomarkers can be found in LB: Tumor-derived markers originating from the tumor itself and tumor-related markers stemming from the body's response to the tumor. Although tumor-associated biomarker discovery is much more complex, their importance in diagnosis, treatment, and prognosis in GBM is much greater (21). In conclusion, LB is a valuable complementary tool to current clinical strategies in the diagnosis and treatment follow-up processes of GBM, which is gaining more and more importance. CTCs, ccfNAs, EVs, and CPs are tumor-derived biomolecules, and studies on their importance in GBM have accelerated in recent years (5,19,22).

CIRCULATING BIOMARKERS in GBM

Circulating Tumor Cells (CTCs) and Their Association with GBM

The presence of CTCs was first identified by Australian researchers in 1869 (14). CTCs have long been known for their role in clinical applications, including cancer detection, genetic profiling, tumor progression tracking, and tailoring personalized therapies. CTCs are tumor-derived cells released into the bloodstream by primary tumors during their formation or growth, which can spread to distant sites and eventually metastasize (23). These cells demonstrate the metastatic potential of epithelial tumor cells (24). Although CTCs are implicated in the development of metastasis, the exact mechanisms involved in this process are intricate and have not yet been clarified (25). The transition of CTCs from epithelial to mesenchymal phenotypes results in the loss of cell-to-cell adhesion, the acquisition of less differentiated mesenchymal characteristics, enhanced migratory potential of cells, and intravasation into circulation. (5, 14). This process led to CTCs becoming an attractive target for tumor biomarker research aimed at early diagnosis in the 1960s and 1970s (21).

While CTCs offer great potential in diagnosing GBM, their integration into clinical practice faces several challenges. The selectively permeable nature of the BBB, the short 24-hour half-life of CTCs, the physical barrier created by endothelial cells in the blood-vessel barrier for tumor cells to enter the circulation, the hemodynamic forces experienced in the circulation, the lack of growth factors and extracellular matrix support, the attack of host immune system cells that suppress tumor migration, and restriction of the mobility of cells in circulation and their capacity for metastasis as a result of interaction with fibrin networks or platelets make the CTCs intravasation into the circulation even more difficult. Even if millions of CTCs are released into the bloodstream from tumors, the concentration of them remains at ~1-10 cells per 10 mL of blood, or 1 in 109 cells. This explains why CTCs are technically difficult to detect with high specificity and sensitivity (21).

Nevertheless, the potential for investigating CTCs in clinical trials is increasing daily. Recent studies have confirmed the presence of CTCs with glial features in the PB of GBM patients, and it has been shown that the genomic content of these cells accurately represents the tumor of origin (26). CTCs are as common as 75% in GBM (27). These findings place CTCs in a promising position not only as an innovative tool in GBM diagnosis and follow-up but also as a promising alternative to conventional TB. The potential of CTCs to offer the advantages of biopsy to a broad patient population in a minimally invasive approach further increases the clinical value of LB applications (28).

A study by Müller et al. showed that CTCs were present in the blood of 29 of 141 (20.6%) GBM patients by immunochemical analysis using immunostaining of mononuclear cells enriched with antibodies against glial fibrillary acidic protein (GFAP). In addition, the presence of CTCs in PB was evaluated prior to and following surgical resection. CTCs were found in both pre- and post-surgical samples in 13.4% of patients, only in post-surgical samples in 7.5%, and only in pre-surgical samples in 6% (27).

CTCs may also be helpful in monitoring the response to treatment in GBM patients. In a study conducted by Gao et al. on 31 patients with seven different pathologic types of primary glioma at WHO stages II, III, and IV, the incidence and number of CTCs in the PB of patients preoperatively and 1 week postoperatively were determined. CTCs were observed in the blood of 24 of 31 (77.4%) patients with primary glioma and 9 of 11 (81.8%) patients with GBM. The researchers reported that the CTC counts of postoperative patients decreased significantly compared to pre-treatment levels. When postoperative CTC counts were analyzed, it was found that CTC counts decreased in 19 of 24 (79.2%) patients with primary glioma and 7 of 9 (77.8%) patients with GBM who had CTCs detected in their blood before the operation. They concluded that detecting of CTCs may contribute to differentiating of radiation necrosis from actual tumor progression (29). In another study supporting these findings, CTCs were

identified in 72% of patients with GBM, and this rate decreased to 8% after RT. The detection of CTCs was performed with a method based on telomerase activity, taking advantage of the high sensitivity and specificity provided by the high telomerase expression seen in over 90% of solid tumors despite the absence of telomerase expression in normal cells. The results of the study show that telomerase activity-based strategies have significant potential for evaluating treatment response and monitoring disease recurrence in patients receiving RT (30). In a retrospective analysis of 22 patients who had tumor resection followed by RT and subsequently developed new mass lesions on MRI, the number of CTCs was significantly higher in the tumor recurrence group compared to the tumor necrosis group (31). Sullivan et al. reported the presence of CTCs in 13 PB samples from 33 (39%) GBM patients at different stages of treatment (32).

The reported detection rates of CTCs in GBM patients vary significantly in the literature, ranging from 20% to 75%. This significant variability across studies can be attributed to differences in detection methodologies, patient selection criteria, cohort characteristics, and blood sample collection timing. Immunostaining techniques like GFAP-based enrichment yield lower detection rates compared to PCR-based approaches and microfluidic platforms, which offer higher sensitivity but may also capture non-tumor-derived circulating cells. Additionally, the classification of study participants, whether newly diagnosed or recurrent GBM patients, can substantially influence CTC detection rates. Beyond methodological differences, patient-specific factors such as tumor stage, variations in BBB integrity, and systemic inflammation can also affect the release of CTCs into circulation, thereby impacting their detectability. Similarly, the timing of biological liquid sampling, whether collected pre- or post-treatment, further contributes to the broad range of reported detection rates.

CTCs can circulate as single cells or homotypic/heterotypic clusters showing higher metastatic potential (5, 7). It has been reported that CTCs form clusters with white blood cells (WBCs), and even the presence of CTC-WBC clusters indicates poor prognosis in some tumors, such as hepatocellular carcinoma (33,34). Szczerba et al. concluded that CTC-neutrophil clusters injected into tumor-free mice accelerated tumor formation, increased metastatic potential, and shortened OS as compared with single CTCs (34).

In GBM management, CTCs should provide concrete evidence to improve the efficacy of therapeutic strategies and have a meaningful impact on the disease course. Currently, the presence of CTCs is considered a potential tool detecting prognostically important genetic biomarkers for GBM, such as isocitrate dehydrogenase (IDH) mutations, although CTCs alone are not of sufficient clinical value. However, including CTCs in a broader panel of biomarkers for GBM patients could contribute significantly to diagnostic accuracy and clinical utility.

Circulating Cell-Free Nucleic Acids (ccfNAs) and Their Association with GBM

Cells can release their nucleic acids into circulation. The presence of ccfNAs was first detected in 1948 by Mandel and Metais in the PB of healthy individuals and patients diagnosed with various metabolic and/or oncological diseases (35). Circulating cell-free DNA (ccfDNA) consists of small DNA fragments, approximately 180-200bp in length, released under physiological and pathological conditions and thought to originate mainly from apoptotic cells (36). The ccfDNA originating from normal cells is usually derived from genomic DNA released during apoptosis or inflammation, and its concentration in the blood is low as it is rapidly removed by phagocytes (37). When phagocytic removal is insufficient in cancer, DNA fragments released from apoptotic and/or necrotic cells of tumor origin accumulate in the circulation. Tumor cells can similarly release different classes of RNAs into the bloodstream, such as protein-coding mRNA, small noncoding microRNAs (miRNAs) of approximately 21-24 nucleotides, and long noncoding RNAs of 200 nucleotides or more. In PB and CSF samples of the GBM patients, circulating cell-free RNAs (ccfRNAs) have been demonstrated, emphasizing their potential as biomarkers for prognosis, diagnosis, and monitoring treatment responses (38).

In 1977, Leon et al. reported higher amounts of ccfDNA in cancer patients than in noncancerous individuals (39). Stroun et al. showed that tumor-associated genetic alterations were found in ccfDNA in cancer patients, and subsequent studies confirmed that neoplastic genomic alterations such as mutations in oncogenes or tumor suppressor genes, microsatellite instability and epigenetic variations can be detected in tumor-derived ccfDNA fragments known as circulating tumor DNA (ctDNA) (40). The demonstration that ccfDNA carries the same molecular information as biopsy samples obtained from tumor tissue has paved the way for ctDNA as a potential biomarker for diagnosing and monitoring cancers (41).

Several studies have identified the presence of ctDNA in some cases with primary CNS tumors, including astrocytoma and oligoastrocytoma. GBM is distinguished from other neoplasms by the low ctDNA concentrations and positive index found in the serum of patients. The proportion of ctDNA among all ccfDNA correlates with tumor burden in advanced-stage solid tumors. ctDNA provides a dynamic reflection of tumor progression and contributes to understanding the mechanism underlying gene mutations and drug resistance in primary tumors (42). In addition, ctDNAs reflect the

molecular profile of tumors, including information on targeted mutations in patients with CNS tumors and drug resistance mechanisms in targeted therapy. Through ctDNA analysis, tumor progression and drug resistance mutations can be identified early (6). This approach has been successfully used to detect specific mutations in adult and pediatric patients with brain tumors. Mutations in genes such as O-6-methylguanine-DNA methyltransferase (MGMT) promoter in astrocytic and oligodendroglial tumors (43, 44), death-associated protein kinase (DAPK) in GBMs (45), phosphatase and tensin homolog (PTEN) in astrocytic tumors and GBMs (43, 46), and epidermal growth factor (EGFR) and IDH (46) in gliomas are examples of ctDNA markers (21). Although TB for histological diagnosis and to obtain information on tumor biomarkers is still valid today, the potential of ctDNA as a biomarker leads to promising approaches in the clinic (47).

ctDNA carries tumor-specific mutations that reflect the mutational characteristics of the primary tumor. Therefore it has significant potential in clinical applications for noninvasive tumor tissue sampling (7). To assess whether ctDNA can facilitate genomic interrogation, Piccioni et al. clinically analyzed data from 419 primary brain tumor patients with a next-generation sequencing panel. The ctDNA mutation rate per patient stratified by histologic subtype was 55% in 222 GBM cases. The researchers report that a biopsy-free option, thanks to ctDNAs, shows promise and could provide a pathway for further advances in genomically matched clinical trials (48). In a study by Lavon et al. evaluating the potential of ccfNA as a noninvasive tool for identifying genetic/epigenetic changes in high-grade astrocytomas and oligodendrogliomas during the disease, loss of heterozygosity (LOH) and/or methylation on chromosomes 1p, 19q and 10q that could identify DNA as tumor-specific was detected in 80.5% of astrocytomas and all oligodendrogliomas. The detection rates of these biomarkers in serum were 51% and 55%, respectively, and the specificity was reported to be around 100%. According to these data, ccfDNA in glial tumors was reported to be informative for both LOH and methylation analysis throughout the progression of the disease (43). In a similar study in 2012, Boisselier et al. first attempted to detect ctDNA-based IDH mutations in glioma and found the mutations in 15 of 25 patients (60%) with mutated tumors. In contrast no mutations were detected in 14 patients with wild-type tumors. Sensitivity increased proportionally with tumor volume, and specificity was 100% (49). In a study by Wang et al., relevant tumor tissues from 89 glioma patients were analyzed for MGMT promoter methylation. It was reported that detection of MGMT promoter methylation in CSF samples (65.0%) showed higher sensitivity compared to serum samples (37.3%) (50).

Studies have shown that ctDNA detection rates are greater in CSF than in plasma and serum. One possible reason for this is that the BBB, even if partially impaired, limits the entry of ctDNA from the primary brain tumor into the bloodstream (51). Despite the promising results, using ctDNA as a biomarker for GBM, in particular, remains challenging. Firstly, ctDNA constitutes 0.1% to 5% of the total ccfDNA, varying according to tumor type, grade, and burden (14). Furthermore, gliomas were among the tumor types with the lowest level of detectable ctDNA. Secondly, ctDNA has a short half-life of less than two hours, requiring rapid processing after sample collection. Thirdly, even if detectable, ctDNA levels in blood are very low in cancer patients, necessitating highly sensitive techniques for its identification and differentiation from normal ccfDNA (52).

Upregulation of miR-21 in plasma (53) and tissues (54) of GBM patients has been reported and shown to be associated with lower OS and tumor grading (55). Wang et al. analyzed plasma from ten GBM patients before and after treatment and identified two miRNAs, miR-128 and miR-342-3p, that were down-regulated in patients compared to controls. miR-128 and miR-342-3p levels were associated with glioma grades and increased following surgery and chemoradiation, indicating their potential as biomarkers for tumor grading and treatment response assessment (56).

To aid molecular diagnosis in GBM, monitor tumor response, identify early recurrence, and follow glioma clonal evolution, although ccfNAs may be helpful as a minimally invasive tool to characterize recurrent tumors and lead to targeted therapies molecularly, there is insufficient evidence for the use of ccfNAs as a biomarker in GBM in routine clinical practice and large prospective studies are still needed to confirm how reliably ccfNAs can reflect the mutational character of GBM, especially when using comprehensive genomic technologies (57).

Circulating Extracellular Vesicles (cEVs) and Their Association with GBM

EVs are small vesicles surrounded by a membrane-bound bilayer lipid membrane secreted into the extracellular space by both healthy and tumor cells under physiological or pathological conditions (6). Structurally composed of various cellular components such as proteins, lipids, and nucleic acids, EVs are heterogeneous in terms of their size, origin, nature, and quantity of molecular content and biological activity and are categorized according to these characteristics. The most widely studied categories of EVs are exosomes, ranging in size from 50 to 150nm, and microvesicles (MVs), ranging from 50 to 1000nm (58). Exosomes are intraluminal vesicles that form into the endosomal membrane during the maturation of multivesicular endosomes (MVEs). The fusion of MVEs with the cell membrane results in the

release of exosomes into the extracellular space (5). MVs are vesicles formed by direct budding of the outer cell membrane (58). Other subclasses of EVs include apoptotic bodies and oncosomes, which are formed nonviably due to apoptosis (59).

EVs mediate intercellular communication (21) and modulate recipient cells' molecular functions by releasing diverse biological factors (6). Therefore, tumor cells secrete exosomes carrying tumor-specific biomarkers, enabling the identification of primary tumor properties. EVs secreted by neoplastic cells can induce the response of neighboring stromal cells with their molecular content, induce direct EV-target cell surface contact by affecting the corresponding membrane-associated receptors, and even alter the program of recipient cells by transferring relevant functions to target cells (60). Pioneering studies have shown that cEVs are critical in generating resistance in GBM. Tumor cells use cEVs to regulate processes such as modulating the tumor microenvironment to promote tumor growth, proliferation, angiogenesis, immune tolerance, drug resistance, modification of tumor metabolism, metastasis, invasion, and avoidance of cell death (61). Exosomes can shape tumor progression, suppress antitumor immunity by promoting angiogenic activity, and accelerate metastatic tumor growth. With these functions, they may contribute to tumor progression. Studies have shown that exosome components largely depend on their initial host cells, suggesting that exosomes carry or mimic the information of their parent cells. Exosomes may represent useful cancer diagnostic biomarkers (62).

Under physiological conditions, tumor cells produce cEVs at a higher rate than normal cells (24). Unlike ctDNAs released from apoptotic cells, cEVs originate from living cancer cells and retain their content from enzymatic degradation (63). cEVs derived from tumor cells are known to be associated with prognosis in many cancers. Mutations of KRAS, EGFR, BRAF, and TP53 genes in DNA in tumor-derived exosomes have been identified in pancreatic, non-small cell lung carcinoma, melanoma, and colorectal cancer, respectively (64). Patient-derived cell lines and cEVs also contain brain tumor markers such as HER2, EGFR, and mutant IDH (65, 66).

The ability to circulate in various body fluids like CSF, urine, and plasma gives cEVs the benefit of being a noninvasive testing alternative. In addition, cEVs are a stable tool for genomic testing as their lipid bilayers protect biomacromolecules such as RNA, DNA, and proteins from enzymatic activity (67). Due to the increased secretion of cEVs by neoplastic cells, they may serve as a rich source of information about GBM's heterogeneous biodiversity, tumor condition, and disease progression (17).

GBM cells have been observed to secrete cEVs that interact with endothelial cells to induce angiogenesis and stimulate tumor cell growth via an autocrine mechanism. Skog et al. provided evidence that cEVs can be obtained from the serum of brain tumor patients and that specific genetic alterations in the EGFR gene can be detected in these cEVs (68). In addition, studies in the serum of GBM patients revealed that different RNA expression profiles can be detected in the cEVs of tumor patients compared to the control group (69). Osti et al. found that plasma concentrations of cEVs were increased in patients with GBM compared to healthy individuals, and this was related with recurrence after tumor resection. In the same study, in order to examine how the cEV proteome is affected by GBM, protein profiles of plasma cEVs obtained from matched GBM patients before and after surgery were extracted, and the expression differences of 102 proteins in the pre- and postoperative groups were shown. It has been suggested that cEVs may be a valuable biomarker to distinguish patients with GBM from other brain injury-related diseases and be useful in early diagnosis (70). Some studies suggest that TMZ treatment may affect cEV release and potentially lead to drug resistance. Analyzing the molecular profile of cEVs may be useful in monitoring the efficacy of TMZ treatment (14). All these features make cEVs an important study area for developing new therapeutic alternatives in glioma (71).

All these clinical data suggest that cEVs may have a potential role in the diagnosis, follow-up, and prognosis of GBM. However, there are also some current limitations. One of the biggest challenges in this field is the lack of standard protocols for cEV enrichment and characterization and the difficulty of cEV research in achieving consistency on a specific standard. This lack of standardized protocols for the isolation, analysis, and reporting of cEVs reduces the comparability of results obtained in different laboratories or studies and leads to complexity (21). In addition, it is still unclear which biosolids are the most appropriate or sufficient source of GBM-derived cEVs. Especially in GBM patients, the permeability of the BBB for cEVs is well known, so plasma, CSF, urine, or saliva may be a more suitable option for cEV fluid biopsy (21). In addition, a limited number of studies demonstrate the isolation and characterization of cEVs from a large number of complex specimens. Studies with larger cohorts are needed to clinically validate the cEVs potential role and see whether they can distinguish GBM from other brain tumors (7).

Circulating Proteins (CPs) and Their Association with GBM

CPs detectable in serum have been widely studied for their potential as biomarkers for many types of cancer. However, since no GBM-specific protein has been

identified so far, studies on detecting changes in the levels of some proteins released into the circulation, specifically from GBM tumor cells, have gained momentum (26). Finding a protein with biomarker potential is very important in diagnosing and monitoring response to treatment, especially in aggressive tumors such as GBM. As a result of studies conducted in this context, proteins such as immunosuppressive acidic protein (IAP), alpha-1 acid glycoprotein (AGP), alpha-1 antitrypsin (AAT), fibronectin and thrombomodulin-1 (TM-1) stand out among the protein biomarkers detected for the first time in the blood plasma of patients with brain tumor (72). However, due to the aggressiveness and highly angiogenic nature of GBM, the search for CPs has turned to angiogenesis-related proteins. In a study conducted by Chiorean et al., angiogenesis and inflammation-related vascular endothelial growth factor (VEGF), platelet derived growth factor BB (PDGF-BB), insulin-like growth factor-1 (IGF-1), transforming growth factor beta (TGF-β), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and interleukin-8 (IL-8) levels were measured in preoperative serum samples of 14 GBM patients and 32 healthy patients. Serum PDGF-BB, IGF-1, and IL-8 levels were increased in GBM compared to the controls. Reduced IL-8 levels were linked to the development of coagulation necrosis, while increased levels with the development of endothelial hyperplasia and elevated VEGF levels were related with the development of ischemic necrosis (73). VEGF serum and tissue levels have been reported to be significantly higher in GBM compared to the controls and even increased in patients with brain metastases (74). It has also been shown that this increase in serum and tissue levels in GBM is due to increased VEGF gene expression (74). One another CP showing high expression in GBM compared to healthy brain tissues is chitinase-3-like protein 1 (YKL-40). A meta-analysis study to determine its prognostic value in GBM was found that elevated YKL-40 expression was associated with a worse OS in patients. It was concluded that it may be a good predictive tool as a prognostic biomarker for GBM patients (75).

GFAP is the protein most commonly detected in GBM and shows high expression levels. It is considered an immunohistochemical marker, especially in determining whether the tumors have glial character. These proteins, found in the cytoplasm of astrocytes, function in myelinization and astrocyte-neuron connection as glial intermediate filaments and are the most valuable indicator for neoplastic astrocytomas (76). Serum GFAP levels have been reported to correlate with tumor volume and histopathological tumor characteristics (77). In the study by Pérez-Larraya et al., preoperative plasma levels of insulin-like growth factor-binding protein 2 (IGFBP-2), YKL-40, and GFAP were measured in GBM patients. The diagnostic and prognostic values of IGFBP-2, GFAP, and YKL-40, both alone and as a combined biomarker profile, have been investigated, and it has been reported that a biomarker profile consisting of preoperative IGFBP-2, GFAP, and YKL-40 levels may be a helpful tool in the diagnosis of inoperable brain lesions with suspected GBM. In addition, it was concluded that IGFBP-2 levels can be considered an independent prognostic factor in GBM patients (78).

Determining the biomarker properties of proteins released into the circulation by GBM cells is of great importance for diagnosing the disease, monitoring response to treatment, and detecting relapses. However, the fact that the content of CPs varies depending on the character and localization of the tumor and their low concentrations makes detecting these proteins difficult. For these reasons, a standard biomarker for clinical use has not yet been defined. Therefore, further studies identifying more sensitive and specific CPs specific to GBM are critical in diagnostic processes and prognostic evaluations.

Limitations of the Review

This review comprehensively examines circulating biomarkers in GBM, but several limitations should be acknowledged. The review predominantly relies on existing literature, which may include studies with limited sample sizes and varying methodologies, leading to inconsistencies in findings. The heterogeneity of GBM and the complexity of biomarker analysis present challenges in drawing generalized conclusions. Moreover, the lack of large-scale clinical trials and standardized protocols for biomarker detection further restricts the ability to provide definitive recommendations for clinical practice. Additionally, while the review discusses various biofluids, a more focused comparison of their diagnostic utility would have strengthened the analysis.

Strengths of the Review

This review successfully highlights the transformative potential of circulating biomarkers in GBM diagnosis and monitoring. One of its major strengths lies in the comprehensive coverage of different biomarker types, including CTCs, cfNAs, cEVs, and CPs. Integrating preclinical and clinical evidence provides a well-rounded perspective on their current and future applications. Furthermore, the review emphasizes the clinical challenges and technical barriers, offering valuable insights into areas that require further research. The detailed exploration of emerging technologies and novel approaches adds depth and relevance, making this review a valuable resource for researchers and clinicians.

CONCLUSION and FUTURE PERSPECTIVES

The high incidence and mortality rates of brain tumors make the development of minimally invasive techniques

for the diagnosis and follow-up of both primary and metastatic tumors an urgent necessity. Despite significant developments in understanding the pathogenesis of GBM, patients still face low survival rates and limited treatment options. The current diagnostic process of GBM relies heavily on imaging modalities and TBs. However, this standard protocol has several limitations, including the inability to accurately represent the tumor, to assess tumor activity in real-time, and the surgical risks of repeated biopsies.

LBs offer many advantages over existing approaches. In particular, they provide reproducible sampling with a noninvasive method and allow tumor-associated molecules to enter the circulation in cases of increased permeability of the BBB. LBs have shown promise in the diagnosis and prognostic evaluation of GBM by providing valuable information before the clinical progression of the tumor. Blood, CSF, urine, and other body fluids carry tumor-associated biomarkers, including CTCs, ccfNA, EVs, and CPs. Studies in the literature show that these biomarkers are present in GBM patients, and their mutation profiles represent the origin of the tumor. This review provides a comprehensive summary of the available literature evaluating the role of biomarkers in the pre-diagnostic process and in monitoring response to treatment. It also sheds light on future research areas for discovering and validating GBM-specific biomarkers.

Integrating biomarkers with genetic and molecular profiling analyses is considered an important step toward more detailed monitoring of tumors. Current research suggests that the features of GBM that develop resistance to treatment can be better predicted by evaluating genetic mutations and biomarkers. In recent years, the correlation of ccfDNAs and ccfRNAs with tumor size and the tumor biology-reflective properties of EVs and CPs suggest that these biomarkers are promising tools for diagnosis and monitoring response to treatment. In particular, the presence of high levels of certain microRNAs associated with high-grade gliomas in treatment-resistant patients is valuable as a prognostic tool in the clinical management of GBM patients. However, several challenges remain that limit the clinical utilization of circulating biomarkers. These challenges include low concentrations of biomarkers, lack of standardized sampling and analysis methods, and the need to improve the specificity and sensitivity of biomarker detection. In the future, to overcome these challenges, larger-scale validation studies, standardization of detection techniques, and prospective studies to develop economically feasible methods should be conducted.

DECLARATIONS

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Review

What's Missing In Diabetes Treatment? A Novel Agent Finerenone?

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Abstract

Diabetic kidney disease represents a leading cause of chronic kidney disease and end-stage renal disease worldwide. The pathogenesis is primarily driven by persistent hyperglycemia, which induces oxidative stress, low-grade chronic inflammation, and activation of profibrotic signaling pathways. These mechanisms promote mesangial expansion, podocyte injury, and tubular epithelial-to-mesenchymal transition, culminating in glomerulosclerosis and tubulointerstitial fibrosis. Fibrosis is a hallmark of progressive diabetic kidney disease, characterized by excessive deposition of extracellular matrix components, leading to structural distortion and progressive decline in glomerular filtration rate.

Proteinuria, a key clinical manifestation of diabetic kidney disease, reflects dysfunction of the glomerular filtration barrier and serves as both a marker and mediator of disease progression. Filtered proteins exert direct cytotoxic effects on proximal tubular epithelial cells, inducing proinflammatory and profibrotic responses that exacerbate tubulointerstitial injury and accelerate fibrosis.

Despite standard-of-care therapy with renin-angiotensin-aldosterone system blockade, a significant proportion of patients exhibit residual proteinuria and progressive renal fibrosis, underscoring the need for additional therapeutic interventions. Mineralocorticoid receptor overactivation has emerged as a critical driver of renal inflammation and fibrosis in diabetic kidney disease. Finerenone, a novel non-steroidal, selective mineralocorticoid receptor antagonist, has demonstrated potent antifibrotic and antiproteinuric effects by attenuating the transcription of proinflammatory and profibrotic mediators, including transforming growth factor-beta and connective tissue growth factor. Finerenone reduces macrophage infiltration, extracellular matrix accumulation, and fibrosis in glomerular and tubulointerstitial compartments.

The landmark FIDELIO-DKD and FIGARO-DKD trials established the efficacy of finerenone in reducing albuminuria and slowing the progression of kidney disease in patients with type 2 diabetes and chronic kidney disease. By directly targeting key pathophysiological mechanisms of renal fibrosis and proteinuria, finerenone offers a novel and evidence-based therapeutic strategy to mitigate kidney disease progression in this high-risk population.

Keywords: Finerenone, Diabetes Mellitus, Nephropathy, Proteinuria, Hyperkalemia

INTRODUCTION

Diabetes mellitus is a complex metabolic disease with a high global prevalence and represents a leading cause of chronic kidney disease (CKD) and cardiovascular morbidity and mortality. Despite intensive glycemic control, many patients experience progressive microvascular and macrovascular complications, including diabetic nephropathy and cardiovascular disease, which substantially contribute to increased mortality and morbidity rates (1,2). Traditional management strategies have focused primarily on glycemic control through the use of oral antidiabetic agents and insulin therapy. However, although these approaches effectively reduce hyperglycemia, they often fail to prevent or adequately slow the progression of diabetes-related end-organ complications, particularly renal and cardiovascular outcomes (3).

The pathophysiology of diabetic complications

is multifactorial and involves chronic low-grade inflammation, oxidative stress, and endothelial dysfunction. These mechanisms drive the progression of both microvascular complications—such as diabetic nephropathy and retinopathy—and macrovascular complications, including ischemic heart disease and stroke (1,2). In particular, persistent oxidative stress and inflammation activate fibrotic signaling pathways that contribute to mesangial matrix expansion, podocyte injury, and tubulointerstitial fibrosis, ultimately resulting in glomerulosclerosis and progressive decline in glomerular filtration rate (4).

Sodium-glucose cotransporter-2 (SGLT-2) inhibitors have emerged as a cornerstone therapy in diabetic kidney disease, offering significant cardio-renal protection independent of glycemic control. These agents have been shown to reduce the risk of hospitalization for heart failure and slow the progression of CKD, irrespective of preexisting atherosclerotic cardiovascular disease or heart failure history (5). Current guidelines recommend initiating SGLT-2 inhibitors in patients with type 2 diabetes mellitus and albuminuria—defined as a urine albumin-to-creatinine ratio (UACR) >300 mg/g—particularly when estimated glomerular filtration rate (eGFR) is greater than 30 mL/min/1.73 m², to reduce the risk of both cardiovascular events and CKD progression (6).

Nevertheless, despite optimized renin-angiotensin-aldosterone system (RAAS) blockade and SGLT-2 inhibitor therapy, many patients exhibit persistent albuminuria and progressive kidney fibrosis, highlighting the need for additional therapeutic interventions. One important mechanism contributing to this residual risk is the phenomenon of aldosterone breakthrough, whereby chronic RAAS blockade becomes insufficient to suppress plasma aldosterone levels. Elevated aldosterone promotes overactivation of mineralocorticoid receptors, which plays a central role in mediating renal inflammation, oxidative stress, and fibrogenesis (7,8).

Mineralocorticoid receptor antagonists (MRAs) have demonstrated efficacy in attenuating these pathological processes by blocking mineralocorticoid receptor activation. MRAs inhibit the transcription of key pro-inflammatory and pro-fibrotic mediators, including transforming growth factor-beta (TGF-β), connective tissue growth factor (CTGF), osteopontin, platelet-derived growth factor (PDGF), plasminogen activator inhibitor-1 (PAI-1), and CC-chemokine ligand 2 (CCL2). This results in reduced macrophage infiltration, decreased collagen deposition, and suppression of extracellular matrix accumulation within both glomerular and tubulointerstitial compartments (9). In addition, MRAs have been shown to attenuate fibroblast activation, reactive oxygen species generation, mesangial expansion, and glomerular hypertrophy—key pathological features of diabetic nephropathy (10).

Clinical studies have established the role of MRAs as adjunctive therapy to RAAS inhibitors and SGLT-2 inhibitors in diabetic nephropathy. Beyond their nephroprotective effects, MRAs confer additional cardiovascular benefits, particularly in reducing the risk of hospitalization for heart failure, a common comorbidity among diabetic patients (11).

Among MRAs, finerenone has emerged as a novel non-steroidal selective antagonist with high affinity for the mineralocorticoid receptor. Finerenone exhibits a more favorable safety profile compared to traditional steroidal MRAs, such as spironolactone, with a lower incidence of hyperkalemia and fewer off-target effects, including gynecomastia (12,13). These properties make finerenone a suitable option for patients with diabetic nephropathy and reduced kidney function. Current guidelines recommend the use of finerenone in patients with diabetic nephropathy who are at high risk of cardiovascular events or CKD progression, particularly when serum potassium levels are ≤4.8 mmol/L or in cases where SGLT-2 inhibitors are contraindicated or not tolerated (14).

Finerenone has demonstrated significant reductions in albuminuria and slowed CKD progression in patients with type 2 diabetes mellitus and albuminuric CKD, as evidenced by the FIDELIO-DKD and FIGARO-DKD trials (15,16). Through its potent anti-inflammatory and anti-fibrotic effects, finerenone offers a mechanistically distinct and evidence-based approach for reducing residual albuminuria and mitigating renal fibrosis in diabetic kidney disease.

METHODS

This review was designed as a systematic narrative review following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The objective was to comprehensively evaluate the efficacy, safety, and clinical application of finerenone, a non-steroidal mineralocorticoid receptor antagonist, in patients with type 2 diabetes mellitus and chronic kidney disease, with an emphasis on renal and cardiovascular outcomes.

Data Sources and Search Strategy

A comprehensive search of the literature was conducted using four major electronic databases:

- PubMed/MEDLINE
- Embase
- Scopus
- Cochrane Library

The search was performed for studies published from January 2010 to March 2025, using a combination of controlled vocabulary (MeSH terms) and free-text

keywords. The following search terms were used in various combinations:

"Finerenone", "Mineralocorticoid receptor antagonist", "Non-steroidal MRA", Diabetic kidney disease", "Chronic kidney disease", "Albuminuria", "Proteinuria" "Cardiorenal outcomes", "Cardiovascular disease", "Heart failure", "SGLT-2 inhibitors", RAAS blockade"

Inclusion Criteria

- Study type: Randomized controlled trials (RCTs), meta-analyses, systematic reviews, and large observational studies.
- Population: Adult patients (≥18 years) with type 2 diabetes mellitus and chronic kidney disease (CKD), with or without cardiovascular disease.
- Interventions: Studies evaluating finerenone as monotherapy or in combination with other agents (e.g., RAAS blockers, SGLT-2 inhibitors).
- Outcomes: Studies reporting renal endpoints (e.g., eGFR decline, albuminuria/proteinuria reduction, end-stage renal disease) and/or cardiovascular outcomes (e.g., cardiovascular death, hospitalization for heart failure).

Exclusion Criteria

- Case reports, case series, editorials, letters to the editor, conference abstracts, and non-peer-reviewed articles.
- Preclinical or animal studies not directly translatable to clinical outcomes.
- Studies exclusively focused on non-diabetic kidney disease, unless relevant to mineralocorticoid receptor mechanism of action.

RENAL OUTCOMES

The renoprotective effects of finerenone have been clearly demonstrated in two pivotal, large-scale, randomized, double-blind, placebo-controlled trials: the Finerenone in Reducing Kidney Failure and Disease Progression in Diabetic Kidney Disease (FIDELIO-DKD) study and the Finerenone in Reducing Cardiovascular Mortality and Morbidity in Diabetic Kidney Disease (FIGARO-DKD) study (14,15) (**Table 1**).

The FIDELIO-DKD trial was specifically designed to evaluate the impact of finerenone on renal and cardiovascular outcomes in patients with type 2 diabetes mellitus and chronic kidney disease. The study enrolled patients with stage 3 to 4 chronic kidney disease, defined by an estimated glomerular filtration rate (eGFR) of 25 to 60 mL/min/1.73 m² and a urine albumin-to-creatinine ratio (UACR) greater than 300 mg/g, all of whom were receiving optimized renin-angiotensin-aldosterone system (RAAS) blockade therapy with either an angiotensin-converting enzyme inhibitor (ACEi) or an angiotensin receptor blocker (ARB). Over a median follow-up period of 2.6 years, finerenone significantly reduced the risk of the primary composite renal outcome, which included a sustained ≥40% decline in eGFR from baseline, progression to end-stage renal disease (ESRD), or renal death, by 18% compared to placebo (14). In addition, finerenone produced a substantial reduction in albuminuria, as evidenced by a significant decrease in UACR levels when compared with placebo (14).

The FIGARO-DKD study extended these findings by enrolling patients with earlier stages of chronic kidney disease (CKD stages 1 to 4) and a broader range of albuminuria (UACR 30 to 5000 mg/g). Over a median follow-up of 3.4 years, finerenone demonstrated a significant reduction in the primary composite cardiovascular outcome, which included cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, or hospitalization for heart failure (15). Although cardiovascular outcomes were the primary focus of FIGARO-DKD, the trial also confirmed finerenone's renoprotective benefits as a key secondary endpoint, particularly in patients with more severe albuminuria (15).

In the FIDELIO-DKD study, finerenone was associated with a clear slowing of eGFR decline and a meaningful reduction in albuminuria. However, an increased incidence of hyperkalemia was reported compared to placebo, though this was generally manageable with regular monitoring and dose adjustments (14). Similarly, in the FIGARO-DKD trial, while the incidence of hyperkalemia was higher in the finerenone group, the rates of treatment discontinuation due to hyperkalemia remained low (15).

Secondary renal outcome analyses from FIGARO-DKD demonstrated that finerenone reduced the risk of ESRD

Table 1. Renoprotective effects of finerenone

Study	Population	Primary Renal Endpoint	Outcome (Risk Reduction)
FIDELIO-DKD	Type 2 Diabetes + CKD Stages 3-4, UACR >300 mg/g	≥40% eGFR decline, ESRD, or renal death	18% relative risk reduction in renal outcomes (HR 0.82)
FIGARO-DKD	Type 2 Diabetes + CKD Stages 1-4, UACR 30-5000 mg/g	Secondary outcome: ESRD or sustained eGFR decline by 40%, renal death	36% reduction in progression to ESRD in patients with severely increased albuminuria
FIDELITY (Pooled Analysis)	Combined population from FIDELIO-DKD and FIGARO-DKD	Composite of renal outcomes	Consistent renal benefit across CKD stages (HR 0.86)

CKD; Chronic Kidney Disease, **UACR;** Urine Albumin-to-Creatinine Ratio, **eGFR;** Estimated Glomerular Filtration Rate, **ESRD;** End-Stage Renal Disease, **HR;** Hazard Ratio.

progression by 36% in patients with severely increased albuminuria, and also provided significant benefits in those with moderately increased albuminuria, with the greatest effect observed in patients with higher baseline albuminuria levels (16).

In patients already receiving RAAS blockade, finerenone was shown to further reduce UACR levels and slow the progression of renal disease, while exhibiting a lower incidence of hyperkalemia compared to steroidal mineralocorticoid receptor antagonists (MRAs) such as spironolactone (13,17). A slight decline in eGFR is commonly observed following initiation of MRAs; however, in the Mineralocorticoid Receptor Antagonist Tolerability Study (ARTS), finerenone was associated with smaller reductions in eGFR and a lower incidence of worsening renal function compared to spironolactone (11,17).

Mineralocorticoid receptor overactivation is a key driver of inflammation and fibrosis in the diabetic kidney. By selectively blocking these receptors, MRAs mitigate these pathogenic processes. Finerenone's high affinity for the mineralocorticoid receptor, coupled with its non-steroidal structure, enhances its anti-fibrotic and anti-inflammatory efficacy while minimizing off-target effects (13).

Both the FIDELIO-DKD and FIGARO-DKD trials required patients to be on maximally tolerated doses of ACE inhibitors or ARBs for at least four weeks prior to enrollment, ensuring that the demonstrated benefits of finerenone were additive to optimized RAAS blockade (14,16).

The FIDELITY pooled analysis of the FIDELIO-DKD and FIGARO-DKD studies reinforced these findings by demonstrating that finerenone provided consistent renoprotective effects across overlapping stages of CKD in patients with type 2 diabetes mellitus. This analysis confirmed reductions in albuminuria and slowed progression to kidney failure when compared with placebo (18).

Based on these robust clinical data, the 2022 Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guidelines for Diabetes Management in Chronic Kidney Disease recommend the use of finerenone in patients with an eGFR of at least 25 mL/min/1.73 m², persistent albuminuria (UACR ≥30 mg/g), and normal serum potassium concentrations (≤4.8 mmol/L), despite treatment with the maximum tolerated dose of RAAS inhibitors (8).

CARDIOVASCULAR OUTCOMES

Cardiovascular disease remains the leading cause of morbidity and mortality in patients with type 2 diabetes mellitus and chronic kidney disease (CKD). Persistent hyperglycemia, oxidative stress, and chronic low-grade inflammation contribute to accelerated atherosclerosis, endothelial dysfunction, and myocardial fibrosis, which collectively increase the risk of cardiovascular events in this population. Despite optimized glycemic control and renin-angiotensin-aldosterone system (RAAS) blockade, substantial residual cardiovascular risk persists, necessitating novel therapeutic interventions that address the underlying pathophysiological mechanisms (1,2) (Table 2).

Mineralocorticoid receptor (MR) overactivation plays a central role in the development and progression of cardiovascular disease in patients with diabetic kidney disease. Excess aldosterone activity promotes myocardial fibrosis, vascular inflammation, and remodeling, thereby contributing to increased arterial stiffness, left ventricular hypertrophy, and heart failure. Mineralocorticoid receptor antagonists (MRAs) attenuate these processes by inhibiting MR-mediated transcription of proinflammatory and profibrotic mediators, such as transforming growth factor-beta (TGF-β), connective tissue growth factor (CTGF), and osteopontin, thereby reducing myocardial fibrosis and improving vascular compliance (5,7).

Finerenone, a novel, selective, non-steroidal MRA, has demonstrated significant cardiovascular benefits in large randomized controlled trials. In the Finerenone in Reducing Cardiovascular Mortality and Morbidity in Diabetic Kidney Disease (FIGARO-DKD) trial,

Table 2. Cardioprotective effects of finerenone

Study	Population	Primary Cardiac Outcomes	Risk Reduction	Follow-up, years
FIGARO-DKD	Type 2 Diabetes + CKD Stages 1-4, UACR 30-5000 mg/g	Cardiovascular death, nonfatal MI, nonfatal stroke, HF hospitalization	13% reduction in cardiovascular events (HR 0.87)	3.4
FIDELIO-DKD	Type 2 Diabetes + CKD Stages 3-4, UACR >300 mg/g	Secondary outcome: Composite cardiovascular events	14% reduction in CV events (HR 0.86) (secondary endpoint)	2.6
FIDELITY (Pooled Analysis)	Combined population from FIDELIO-DKD and FIGARO-DKD	Composite of CV death, nonfatal MI, stroke, HF hospitalization	14% reduction in CV events (HR 0.86), primarily driven by lower HF hospitalization rates	Varied

CKD; Chronic Kidney Disease, **UACR;** Urine Albumin-to-Creatinine Ratio, MI; **Myocardial Infarction, HF;** Heart Failure, **CV;** Cardiovascular, **HR;** Hazard Ratio.

finerenone significantly reduced the risk of the primary composite cardiovascular outcome—defined as cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, or hospitalization for heart failure—by 13% compared to placebo (hazard ratio [HR], 0.87; 95% confidence interval [CI], 0.76–0.98; p=0.03) (15). Notably, the benefit was consistent across different baseline estimated glomerular filtration rate (eGFR) and urine albumin-to-creatinine ratio (UACR) categories, including patients with earlier stages of CKD and moderately increased albuminuria.

In the Finerenone in Reducing Kidney Failure and Disease Progression in Diabetic Kidney Disease (FIDELIO-DKD) trial, finerenone also reduced the risk of cardiovascular events as a key secondary endpoint, with a hazard ratio of 0.86 (95% CI, 0.75–0.99), further confirming its cardioprotective potential in patients with more advanced CKD and higher levels of albuminuria (14).

The FIDELITY pooled analysis, which combined data from both FIDELIO-DKD and FIGARO-DKD, reinforced these findings by demonstrating that finerenone significantly reduced the risk of cardiovascular events by 14% compared to placebo across a broad population of patients with type 2 diabetes mellitus and CKD (hazard ratio [HR], 0.86; 95% CI, 0.78–0.95) (18). The reduction in cardiovascular risk was primarily driven by a lower incidence of hospitalization for heart failure, reflecting the antifibrotic effects of finerenone on the myocardium and its capacity to mitigate cardiac remodeling (18).

Steroidal MRAs such as spironolactone and eplerenone have long been established in the treatment of heart failure with reduced ejection fraction (HFrEF), demonstrating reductions in mortality and heart failure hospitalizations (19,20). However, their use in patients with CKD is often limited by the increased risk of hyperkalemia and worsening kidney function. Finerenone, due to its non-steroidal structure, balanced distribution in cardiac and renal tissues, and higher selectivity for the mineralocorticoid receptor, provides a favorable safety profile with a lower incidence of hyperkalemia compared to traditional MRAs (13,17).

The KDIGO 2022 Clinical Practice Guidelines for Diabetes Management in Chronic Kidney Disease recommend finerenone for patients with type 2 diabetes mellitus, persistent albuminuria (UACR ≥30 mg/g), and high cardiovascular risk, particularly in those who cannot tolerate or are ineligible for sodium-glucose cotransporter-2 (SGLT-2) inhibitor therapy (8). In addition, current heart failure guidelines recommend SGLT-2 inhibitors as first-line therapy for patients with heart failure with preserved ejection fraction (HFpEF). In cases of persistent symptoms despite optimized therapy, the addition of finerenone may be considered, particularly in patients at risk for progressive cardiorenal dysfunction (19,20).

Beyond its established role in reducing heart failure hospitalizations, finerenone may also provide long-term cardiovascular protection by preventing myocardial fibrosis and reducing arterial stiffness—two key mechanisms underlying heart failure with preserved and reduced ejection fraction (21). Ongoing studies are evaluating the potential benefits of combining finerenone with other cardioprotective agents, including SGLT-2 inhibitors and glucagon-like peptide-1 (GLP-1) receptor agonists, in patients with diabetes and cardiorenal syndromes (9,19,22).

SAFETY and ADVERSE OUTCOMES

Finerenone binds to mineralocorticoid receptors with better selectivity and higher affinity and has non-steroidal structure. As a result, the side effect profile is better compared to other MRA. Additionally, different from steroidal MRA, it displays more balanced distribution in cardiac and renal tissues (23).

As with other MRA, hyperkalemia may be observed linked to finerenone. As a result, regular monitoring of serum potassium levels is required. But the hyperkalemia risk is lower compared to spironolactone and generally is at tolerable levels (17). In the FIDELIO-DKD study, hyperkalemia was observed after finerenone use that required lower rates of drug cessation relative to other

Table 3. Comparison of adverse events among MRAs

Parameter	Finerenone	Steroidal MRAs (Spironolactone / Eplerenone)
Hyperkalemia incidence	10.8% to 18.3% (lower than spironolactone)	Higher hyperkalemia incidence, especially in CKD
Hyperkalemia-related treatment discontinuation	1.7% to 3.2% (lower discontinuation rates vs. steroidal MRAs)	Higher discontinuation rates due to hyperkalemia
Gynecomastia and endocrine-related adverse events	Minimal due to non-steroidal structure	Common (gynecomastia, menstrual irregularities)
Blood pressure effects	Mild reduction in systolic BP, less than spironolactone	Stronger BP lowering effect
Drug interactions	CYP3A4 metabolism, caution with inhibitors/inducers	Less prone to CYP interactions, but broader side effect profile
Use in advanced CKD (eGFR <25 ml/min/1.73m²)	Not recommended (evidence limited in eGFR <25 ml/min/1.73m²)	Not preferred in advanced CKD due to hyperkalemia risk

MRA antagonists and the incidence was reported to vary from 1.7% to 3.2% (14). The hyperkalemia side effect risk with the addition of finerenone to treatment of patients using RAAS blockers was observed at lower rates compared to treatment with two RAAS blockers (14,24). The FIDELITY combined analysis reported that the simultaneous use of SGLT-2 inhibitors in patients using RAAS blocker and finerenone may reduce the hyperkalemia side effect risk (18) (**Table 3**).

The United States Food and Drug Administration (FDA) recommends initiating Finerenone therapy at potassium levels <5 mmol/L. The recommendation of the KDIGO 2022 Clinical Practice Guidelines for Management of Diabetes in Chronic Renal Disease is presented in Table 1 (8).

The reduction in systolic blood pressure and increase in serum aldosterone levels in patients receiving finerenone were lower than observed in patients receiving spironolactone. This situation may be an additional benefit for patients with blood pressure controlled well or at low levels with first-choice antihypertensive drugs like RAAS blockers (17).

Due to the selective structure of finerenone, observation of side effects like gynecomastia and feminization, that may be observed after spironolactone use, is not expected (20). Potential drug interactions may occur with finerenone, which is metabolized mainly by cytochrome P450-3A4 enzyme and binds to protein at high rates (25).

Steroidal MRA are drugs with proven efficacy for treatment of low ejection fraction heart failure and primary hyperaldosteronism. They are effective for refractory hypertension (26). For these indications, finerenone cannot take the place of steroidal MRA (8).

Limitations of The Review

Despite the promising evidence supporting the use of finerenone in patients with diabetic kidney disease and cardiovascular risk, several limitations warrant consideration when interpreting the available data and translating it into clinical practice.

First, the majority of evidence regarding the efficacy and safety of finerenone is derived from randomized controlled trials, specifically the FIDELIO-DKD and FIGARO-DKD studies. Although these trials were well-designed and included large, diverse populations, they primarily enrolled patients with type 2 diabetes mellitus, moderately to severely increased albuminuria, and relatively preserved renal function (eGFR ≥25 mL/min/1.73 m²). As a result, the generalizability of these findings to patients with non-diabetic chronic kidney disease, normoalbuminuric diabetic kidney disease, or those with advanced kidney failure (eGFR <25 mL/min/1.73 m²) remains uncertain.

Second, while finerenone has demonstrated a favorable safety profile compared to steroidal mineralocorticoid receptor antagonists, the risk of hyperkalemia persists. Although manageable in controlled clinical trial settings with frequent monitoring and protocol-driven dose adjustments, real-world data on hyperkalemia incidence and management strategies in broader clinical practice are still limited. Patients at higher risk for hyperkalemia, such as those with advanced CKD, heart failure, or concomitant use of potassium-sparing diuretics, require close monitoring, which may present challenges in routine care.

Third, the long-term outcomes beyond the median follow-up durations of the FIDELIO-DKD (2.6 years) and FIGARO-DKD (3.4 years) trials are not yet known. While these studies demonstrated a reduction in surrogate renal and cardiovascular endpoints, further evidence is needed to confirm the durability of these benefits over longer time horizons, particularly regarding progression to end-stage renal disease and long-term cardiovascular mortality.

Additionally, although the concurrent use of sodium-glucose cotransporter-2 (SGLT-2) inhibitors was allowed in both FIDELIO-DKD and FIGARO-DKD, the proportion of patients receiving combination therapy was relatively low. More data are required to assess the efficacy, safety, and potential synergistic effects of combining finerenone with SGLT-2 inhibitors, glucagon-like peptide-1 receptor agonists, and other emerging therapies in patients with diabetic kidney disease.

Finally, there is limited evidence regarding the use of finerenone in specific subpopulations, such as patients with type 1 diabetes mellitus, elderly patients with frailty, and those with significant comorbidities, including liver disease or malignancy. Further studies are necessary to clarify finerenone's role in these groups and to identify additional biomarkers for predicting treatment response and safety.

CONCLUSION

Finerenone represents a significant advancement in the management of patients with type 2 diabetes mellitus and chronic kidney disease. By selectively antagonizing the mineralocorticoid receptor, finerenone addresses key pathophysiological mechanisms—namely inflammation and fibrosis—that drive the progression of diabetic kidney disease and contribute to cardiovascular morbidity and mortality. Robust evidence from the FIDELIO-DKD and FIGARO-DKD trials demonstrates that finerenone reduces albuminuria, slows the decline in estimated glomerular filtration rate, and lowers the risk of cardiovascular events, including hospitalization for heart failure.

Compared to traditional steroidal mineralocorticoid receptor antagonists, finerenone offers a more favorable safety profile, with a reduced incidence of hyperkalemia and fewer endocrine-related adverse effects. These features make finerenone a valuable therapeutic option, particularly for patients who remain at high residual cardiorenal risk despite optimized renin-angiotensin-aldosterone system blockade and, where appropriate, the use of sodium-glucose cotransporter-2 inhibitors.

Current clinical practice guidelines endorse finerenone as an adjunctive therapy in patients with diabetic kidney disease, persistent albuminuria, and high cardiovascular risk. However, further research is needed to confirm its long-term efficacy and safety in broader patient populations, explore its role in combination therapy with other novel agents, and clarify its potential benefits in non-diabetic kidney disease.

In summary, finerenone offers a novel, mechanism-driven approach to cardiorenal protection in patients with type 2 diabetes mellitus and chronic kidney disease, with the potential to improve clinical outcomes and fill an important therapeutic gap in this high-risk population.

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Letter to Editor

Comment On: Pregnancy and The Kidneys: A Brief Systematic Review

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Dear Editor;

In the valuable article titled "*Pregnancy and The Kidneys: A Brief Systematic Review*" in the first issue of the Journal of European Internal Medicine Professionals (2023), the author highlights the importance of preeclampsia, urinary tract infections, and the management of dialysis and kidney transplant patients during pregnancy (1). I would like to add a few data points from the perspective of obstetrics to these compact review.

Gestational hypertension and preeclampsia are hypertensive disorders that can improve postpartum. Globally, in 2019, 18.08 million cases of preeclampsia were identified, and its prevalence continues to rise. Severe preeclampsia causes 70,000 maternal deaths and approximately 500,000 fetal/neonatal deaths each year (2). I would like to emphasize once again that, while gestational hypertension is characterized by elevated blood pressure, it does not include proteinuria or end-organ dysfunction or symptoms. However, as mentioned in the article, women with gestational hypertension are at risk for developing preeclampsia and should be carefully monitored (3).

In preeclampsia, elevated blood pressure can be accompanied by proteinuria. However, the presence of end-organ dysfunction symptoms in the absence of proteinuria may still lead to the diagnosis of preeclampsia. These symptoms, as described in the article, include thrombocytopenia, pulmonary edema, elevated creatinine and transaminase levels, and neurological and visual symptoms. In severe cases of the disease, these end-organ dysfunctions become more pronounced. The possibility of preeclampsia should not be overlooked in the absence of proteinuria (2,3).

The primary treatment for preeclampsia is delivery. If end-organ dysfunction is present, the severity of the condition is assessed, and the timing of delivery is determined based on gestational age. When pregnancy reaches ≥37 week, if preeclampsia worsens, delivery can be planned. In pregnancies ≥34+0 weeks, delivery is indicated if severe preeclampsia develops. In cases of preeclampsia with stable maternal and fetal conditions, management with close monitoring and expectant management is recommended. In pregnancies under 34 weeks, the decision for delivery is made if maternal and/or fetal conditions are unstable, if the pregnancy is not yet at the lower limit of neonatal viability (<23 weeks), or if labor or active rupture of membranes is detected. Otherwise, expectant management should be preferred if possible (4).

It is known that adverse pregnancy outcomes are increased in chronic kidney disease (CKD). Hypertension, proteinuria, fibrinogen levels >4 g/L, serum albumin levels ≥30 g/L, and uric acid levels >260 mmol/L (~4.4 mg/dL) have been identified as independent risk factors for preeclampsia in most patients with Stage 1 CKD . Women with advanced CKD have been found to have higher risks of preeclampsia, premature birth, and neonatal intensive care unit admission (5). It is known that the kidney plays an important role in the development of preeclampsia and that it can trigger endothelial and placental dysfunction, and conversely, the kidney can be damaged by endothelial dysfunction due to preeclampsia (6).

In conclusion, a multidisciplinary approach involving both nephrology and obstetrics is essential to optimize

the management and outcomes of pregnancy-related hypertensive disorders, particularly in patients with underlying kidney disease.

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