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Investigating olfactory and gustatory dysfunction in patients with restless legs syndrome: A comparative analysis with healthy controls

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Ethics Committee Approval
The study was approved by the Ethics Committee of Haydarpaşa Numune Training and Research Hospital, İstanbul Turkey (Approval No: HNEAH-KAEK-2016-70).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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No conflict of interest was declared by the authors.

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Abstract

Background/Aim: This study aims to examine the correlation between dopamine deficiency and olfactory dysfunction in patients with restless legs syndrome (RLS) and to juxtapose these findings with gustatory function. This comprehension of the relationship may illuminate the pathophysiology of RLS, suggesting possible therapeutic targets.
Methods: We included a total of 100 male participants aged 40 and older, comprising 50 healthy volunteers and 50 patients with RLS. Participants were recruited from the Neurology Clinic at Sultan Abdulhamid Han Training and Research Hospital between September 2016 and April 2017. We objectively assessed olfactory function using the Sniffin' Sticks Test Battery, while gustatory function was evaluated using the Taste Strip test—participants identified the flavors presented to them.
Results: The mean age of the RLS patient group was 52.5 years, while it was 52.7 years for the healthy group. Olfactory test scores were significantly lower in the RLS group compared to the healthy group (P<0.05). Likewise, gustatory test scores were also significantly lower in RLS patients (P=0.032). A strong positive correlation was observed between the olfactory and gustatory test results (r=+0.72), indicating a significant decline in both sensory functions in RLS patients.
Conclusion: This study reveals a significant association between dopamine deficiency, olfactory dysfunction, and impaired taste perception in RLS patients. These findings suggest that RLS may involve underlying neurodegenerative processes affecting sensory perception. Further research is crucial to shed light on RLS mechanisms, which remain partially understood, and guide the development of targeted therapeutic strategies.

Keywords: restless leg syndrome, olfactory dysfunction, gustatory dysfunction, sensory testing

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Introduction

Restless leg syndrome (RLS) is a disorder characterized by an intense, often indescribable dysesthesia in the extremities, predominantly the legs, coupled with an overwhelming urge to move them. This restlessness is particularly noticeable at night, interfering with sleep initiation, and can continue for several hours, intermittently lasting as long as 3–5 h in severe cases. Although RLS symptoms may occasionally affect only one side, they are typically bilateral and symmetrical. As a result, RLS significantly impairs sleep quality and frequently leads to chronic sleep disturbances [1].

The diagnosis of RLS primarily depends on a detailed patient history and extensive physical and neurological examination. The condition often presents with subtle symptoms, making diagnosis difficult; it is estimated that only one in four cases is accurately diagnosed [2]. While some diagnostic methods concentrate on involuntary movements during rest, a conclusive diagnosis is founded on set clinical criteria.

Several hypotheses have been proposed to explain the pathophysiology of RLS, including psychological factors, vascular elements, and peripheral and central nervous system pathologies. Psychological factors are thought to be significant due to the high prevalence of depression and anxiety among RLS patients. The vascular hypothesis suggests that the relief of symptoms through movement – which increases venous flow – results from the accumulation of metabolites in the legs. Peripheral nervous system pathologies are noted in many patients, even when neurological examinations reveal no structural lesions; RLS is also associated with polyneuropathy [3]. Akpınar et al. [4] first suggested central nervous system involvement, observing that dopaminergic hypofunction could help alleviate RLS symptoms with L-dopa [3]. The idea that motor restlessness in RLS stems from a lesion in the diencephalospinal dopaminergic system is supported by the successful outcomes of dopaminergic treatments [5].

Idiopathic Parkinson's disease (IPD) is a progressive neurodegenerative disorder caused by the degeneration of dopamine-producing cells in the brain, leading to motor symptoms like tremors, slowed movements, muscle rigidity, and balance issues. These symptoms typically emerge between ages 40 and 75. The disease often goes undiagnosed for years, given its gradual onset and symptoms overlapping with other neurological conditions. The diagnosis of IPD relies on clinical criteria, as there are no definitive lab or imaging tests [6]. Motor symptoms manifest when the damage to dopamine cells exceeds 60%, progressing from the enteric nervous system to the substantia nigra and cerebral cortex. Non-motor symptoms such as loss of smell, sleep disturbances, and constipation may surface years before motor symptoms, signaling the early onset of the disease [7].

IPD, a neurodegenerative disorder typically presenting between the ages of 45 and 70, is more prevalent in men and characterized by bradykinesia, resting tremor, postural instability, and rigidity. Symptoms are often managed with L-dopa therapy. While IPD and RLS both respond to dopaminergic treatment, contrasting findings – such as dopamine deficiency in IPD and hypermetabolism in the substantia nigra in RLS – complicate a clear link [8]. Studies indicate men with RLS have a higher prevalence of IPD, yet recent research suggests RLS may serve as an early predictor rather than a direct risk factor for IPD [9]. Consequently, we hypothesize that taste and smell disturbances, an early sign of Parkinson's, might serve a similar role as an early indicator for RLS.

Materials and methods

This study encompassed male patients aged over 40 with RLS, who experienced RLS symptoms on 15 or more nights each month. Participants were selected from patients presented at the Neurology Clinic at the Sultan Abdulhamid Han Education and Research Hospital between September 2016 and February 2017, with institutional approval. The exclusion criteria included any condition or treatment known to impair taste or smell, depression as indicated by the Beck Depression Inventory, cognitive impairment assessed by the Mini-Mental State Examination, and a diagnosis of Parkinson's disease.

Testing Protocol

The Sniffin' Sticks Test, a validated olfactory assessment, was used to evaluate olfactory function. Resembling felt-tip pens, Sniffin' Sticks contain various odorants that are released upon cap removal, which allows for a controlled odor presentation. Patients were instructed to abstain from food and drink, except water, for at least 15 min before testing. The tests were conducted in a well-ventilated, low-odor room, with the examiner wearing odorless gloves (MediSense Burghart Sniffin' Sticks, 2016).

Test Procedure

For testing, patients wore a sleep mask, and single-nostril assessments were conducted by occluding one nostril with 3M Microfoam. The test involved holding the pen approximately 2 cm from the open nostril. The protocol consists of three stages:

1. **Threshold Test** – Establishes detection sensitivity using a dilution series,
2. **Discrimination Test** – Differentiates between similar odors,
3. **Identification Test** – Assesses the ability to recognize specific odorants.

Each segment – known as threshold, discrimination, and identification – was conducted with intervals of 3 to 5 min in between.

Threshold Test Methodology

The threshold test determines odor sensitivity via a "staircase" method. This method progressively presents increasing dilutions until the odor can no longer be distinguished. Pens numbered from 1 to 16 contain dilutions, with red-capped pens representing varying concentrations. During each trial, patients are presented with three pens in varying orders. These pens include one pen with the odorant and two with only the solvent. Patients start with the most diluted (or least odorous) sample and try to identify the different odors. For each correctly identified sample, an "X" is marked on the score form. Missed identifications, however, receive a "-". The test continues with sequentially stronger dilutions until the participant can identify the odor twice consecutively. This point establishes a detection threshold milestone, with subsequent milestones being recorded thereafter.

Threshold Test Calculation

In the threshold test, calculations are conducted to ascertain olfactory sensitivity, employing the arithmetic mean of the last four values (from positions four to seven) out of seven established threshold turning points identified by the patient.

Discrimination Test

The discrimination test evaluates the capacity to distinguish between odors. This is done by comparing groups of three presentations (triplets), where two pens have the same odor (non-target), and one pen (target) contains a differing odor. The patient's task is to identify the pen with the unique odor. The test comprises 16 triplets, each labeled with green numbers from 1 to 16. Target pens are identifiable through their green caps. Throughout the testing, patients receive verbal instructions – such as pen numbers – to smell each pen in the triplet and identify the distinct odor in every set.

Identification Test (Detection Test)

The identification test evaluates the patient's capacity to identify everyday odors using a multiple-choice format. In this test, the patient is given a card with four options and must pick the one that accurately corresponds to the smell they perceive. The test consists of 16 distinctive odors, labeled using blue numbers from 1 to 16. Patients are mandated to make a choice even if they struggle with the sense of smell or only perceive a faint odor (forced choice). For example, for item number 9, if the options are "onion, sauerkraut, garlic, carrot", the correct answer would be "garlic".

If the threshold score exceeds 1.0 (indicating the patient can differentiate between but/pea and solvent), the overall test result is obtained by adding up the scores from the threshold, discrimination, and identification tests (TDI). The TDI interpretation is as follows:

- A total TDI score greater than 30 indicates normosmia (normal sense of smell).
- A total TDI score greater than 16 indicates hyposmia (reduced sense of smell).
- A total TDI score of less than 16 indicates functional anosmia (loss of the sense of smell).

Taste Strip Taste Test

The "Taste Strip" test is a validated method for assessing taste perception. During the evaluation, strips infused with various flavors are placed on the patient's tongue while their mouth remains closed, thereby allowing the tongue to move freely. Before the test is conducted, it is crucial to ensure that patients have consumed only water, not smoked, and refrained from eating or chewing gum for at least 1 h. Each strip is intended for single use only.

Following the established protocol, 16 distinct taste strips are presented to patients in a random sequence. This includes both flavored and tasteless (blank - labeled as "U" and "V") strips. For example, an "Unpleasant" strip may be presented at the outset to acquaint the patient with the strip's taste. For a full-mouth evaluation, the taste strip is positioned at the center of the anterior third of the extended tongue. Patients are then instructed to close their mouths and gently move their tongues to prevent the strip from spinning or shifting. Between each taste test, a sip of water is used to cleanse the mouth.

After each strip is placed, patients are asked to identify the flavor they perceive, selecting from the options: "sweet", "sour", "salty", "bitter", or "unpleasant". Their responses are recorded on a summary sheet, with one point awarded for each correct identification. The maximum possible score on the test is 16, representing four correct answers for each taste type. The tasteless strips are not included in the evaluation. By summarizing the results, the overall taste performance and functionality for each specific taste quality can be assessed. Normative values for each taste type are provided in an accompanying table.

This study complied with the ethical standards delineated in the Declaration of Helsinki. The Haydarpaşa Numune Training and Research Hospital Ethics Committee granted ethical approval (Approval No: HNEAH-KAEK-2016-70). Furthermore, all participants provided written informed consent before their inclusion in the study, affirming their voluntary participation and understanding of the sensory testing procedures involved.

Statistical analysis

The statistical analysis was conducted using SPSS (Version 26.0, IBM Corp., Armonk, NY, USA). As the data were non-parametric, the Kruskal-Wallis H test was employed to compare differences in the TDI scores, and taste test scores among the healthy control group, RLS patients treated with dopamine agonists, and RLS patients treated with gabapentin. Whenever applicable, post-hoc analyses were utilized to pinpoint specific group differences. For the correlation analysis between odor and taste test scores, Spearman's rho correlation was used to determine the strength and direction of the relationship. A *P*-value of <0.05 was the threshold for statistical significance for all tests.

Results

Participants and Test Findings

In this study, we used the Sniffin' Sticks Test method to assess olfactory and gustatory function, exploring their potential relationship with RLS. Our participant pool consisted of 100 men, selected specifically to investigate if olfactory impairment could serve as an early indicator of Parkinson's disease. We required participants to be over the age of 40 and report more than 15 symptoms per month. Among the 100 participants, 50 individuals (50%) formed a healthy control group (Table 1).

The average age of the healthy group was 52.7 years, with all participants being male. This group included 12 smokers and 38 nonsmokers. On the other hand, the RLS cohort consisted of 50 male patients, who had an average age of 52.5 years and an average RLS severity score of 23.2. In this group, 18 patients were smokers, while 32 were nonsmokers (Table 1).

Table 1: Sociodemographic data.

	Healthy group	Patients with RLS
Age	52.7 (11.37)	52.5 (10.5)
Male/Female	50/0	50/0
Smoking/Non-smoking	12/38	18/32
HBS severity score	0	23.2 (11.2)
UPDRS	0	0
Total	50	50

Additionally, the 50 RLS patients were grouped into two categories based on their treatment: those undergoing dopamine agonist treatment and those treated with gabapentin. The majority, comprising 40 patients, or 80% of the RLS cohort, were on dopamine agonist therapy, while 10 patients, representing 20%, were treated with gabapentin (Table 1). The average age of all the participants was 52.7±8.1 years, with an average age of 52.2±7.9 years in the dopamine agonist group and 55±7.0 years in the gabapentin group (Table 1).

We conducted the Kruskal-Wallis H test to assess the significance of the decreases in TDI means among the groups. Results revealed a statistically significant reduction in each category (P=0.032). Specifically, TDI results showed that the RLS-PRD patient group had a TDI score of 32.5, while the healthy control group had a score of 37.5. Even though the RLS-PRD score remained within the lower limit of normosmia, the difference was statistically significant. Moreover, the threshold means disclosed an average of 8.3±1 in the healthy group, 8.0±1 in the dopamine agonist group, and a noticeably lower average of 6.4±0.7 in the gabapentin group. These findings underscore a significant decrease in threshold averages among selected participants (Table 2).

Table 2: Results of the difference test for decrease in threshold means.

Group	n	Mean rank	Chi-square	DF	*P*-value
Healthy group	50	73.50	74.668	2	<0.001
Dopamine agonist	40	31.45			
Gabapentin	10	11.70			

The Kruskal-Wallis H test was utilized to assess whether the decrease in mean threshold was significant, given the non-parametric characteristics of the data. We established that the decrease was indeed significant (P<0.001) (Table 2). Discrimination means were determined to be 14.8±0.8 in the healthy group, 12.1±2.3 in the dopamine agonist group, and 11.3±0.7 in the gabapentin group. These findings suggest that discrimination means a decline along with an increase in RLS severity. To confirm the statistical significance of the decrease in discrimination averages, the Kruskal-Wallis H test was re-administered. The results demonstrated significant changes in discrimination averages (P=0.032) (Table 3).

Table 3: Results of the difference test for reduction in identification means.

Group	n	Mean rank	Chi-square	DF	*P*-value
Healthy group	50	68.90	46.932	2	<0.001
Dopamine agonist	40	36.15			
Gabapentin	10	15.90			

The average TDI was 37.5 in the healthy group, 32.6 in the dopamine agonist group, and 28.8 in the gabapentin group. As such, the average TDI decreased proportionally to the increasing severity of RLS. Nonetheless, the average TDI remained at the normosmia level in the dopamine agonist group, while it dropped to the hyposmia level in the gabapentin group (Table 4). The Kruskal-Wallis H test was employed to evaluate the significance of the decline in TDI averages; it showed a significant difference (P<0.001) (Table 4).

Table 4: Results of the difference test for reduction in TDI means.

Group	n	Mean rank	Chi-square	DF	*P*-value
Healthy group	50	72.50	72.250	2	<0.001
Dopamine agonist	40	33.20			
Gabapentin	10	9.70			

Additionally, taste test averages were observed to be 15.4±0.6 in the healthy group, 12.5±2.4 in the dopamine agonist group, and 11.1±1.8 in the gabapentin group. Like the other sensory tests, taste averages decreased with increases in RLS severity. The Kruskal-Wallis H test was employed to determine if this decrease in taste averages was statistically significant. The results indicated a significant decrease (P<0.001) (Table 5).

Lastly, we analyzed whether a correlation existed between the averages of the odor and taste tests. Analysis by Spearman's rho correlation, suitable for non-parametric data, revealed a correlation coefficient of +0.72. This indicates a strong positive correlation between the odor and taste tests (Table 5).

Table 5: Results of the difference test for reduction in taste means.

Group	n	Mean rank	Chi-square	DF	*P*-value
Healthy group	50	71.66	64.005	2	<0.001
Dopamine agonist	40	33.33			
Gabapentin	10	13.40			

Discussion

This study is among the first to directly investigate olfactory and gustatory testing in patients with RLS, shedding light on the interrelationship between these sensory modalities. We hypothesized that neurodegeneration in the olfactory nerve might manifest early in this population. Consequently, we expected olfactory and gustatory impairments to emerge, attributing them to neuronal degeneration associated with RLS. Significantly, our results showed a reduction in olfactory and gustatory test scores among RLS patients, deemed at risk for neurodegenerative conditions. Interestingly, we observed a positive correlation between the findings of both olfactory and gustatory tests, implying that sensory impairments may occur concurrently in this patient group. Hence, these insights critically suggest that olfactory dysfunction could serve as an early indication of neurodegenerative processes in individuals suffering from RLS.

The comparative analysis showed that the average age of RLS patients over 40 was 52.5 years, while the average age of the healthy control group was slightly higher at 52.7 years. The TDI results for the RLS cohort were significantly lower at 32.5 compared to the healthy group's 37.5, even though they still fell within the lower limit of normosmia. This discovery contradicts the literature, as only one previous study by Adler et al. [10] reported olfactory dysfunction in RLS patients. In Adler's research, the patient's average age was 67, with a severity scale score of 8.7, which is higher than the 23.2 in our study. Moreover, while Adler used UPSIT for his study, ours applied the Sniffin' Sticks method, contributing to statistically significant results in our group. These variations bolster the notion that diverse pathophysiological mechanisms might exist among different RLS patient groups.

Furthermore, among the RLS patients, those receiving dopamine agonists had a mean age of 52.2 years, while those in the gabapentin group had a slightly higher mean age of 55 years. This observation aligns with the findings of Beaven et. al. [11] who reported an increased prevalence and severity of olfactory dysfunction with age, and use of gabapentinoid medications. When comparing the identification test averages, a significant reduction was observed: the dopamine agonist group averaged 12.5, and the gabapentin group averaged 11.1. This correlates with the observed decreases in TDI scores, which were 32.6 in the dopamine agonist group and 28.8 in the gabapentin group. While the TDI scores remained within the normosmia range for the dopamine agonist group, the gabapentin group fell into the hyposmia range. These results align with the findings of Doty et al. [12], who emphasized the neurotransmitter-related pathophysiology of olfactory disorders, suggesting that the dopamine agonist group yielded higher TDI scores compared to the gabapentin group.

Additionally, our findings revealed a significant decrease in taste averages, with the dopamine agonist group averaging 13.1, and the gabapentin group averaging 12.1. There was a strong

positive correlation of +0.72 between the TDI and taste test results, suggesting that these sensory modalities might fluctuate together. Although a mechanism underlying taste disorders remains elusive due to the scarcity of related studies, it is known that sweet afferent nerves largely project to the nucleus of the solitary tract (NTS). Notably, the absence of significant pathology in the medullary region among our RLS patients, coupled with the preservation of the NTS, raises questions about the potential influence of medication on taste function [13]. Factors such as poor oral hygiene and changes in salivary consistency may also play a role.

The clinical significance of our findings is considerable. Patients presenting concurrent olfactory and gustatory dysfunctions should be closely monitored for potential neurodegenerative disorders [15]. Regular follow-up visits might be necessary to assess changes in sensory function over time, allowing for early intervention when needed. Our study suggests that the sensory deficits observed in the RLS-PRD group could indicate a shared pathophysiology with Parkinson disease (PD), strengthening our initial hypothesis about the neurodegenerative implications of olfactory dysfunction in this patient population. The occurrence of concurrent sensory deficits in this clinically high-risk group calls for increased vigilance and potential screening for neurodegenerative diseases [16].

Limitations

This study possesses several limitations that must be taken into account. Firstly, the sample included only male participants over 40 years of age, which could limit the generalizability of our findings to other demographic groups, such as women and younger individuals. These groups may experience different patterns of RLS and sensory dysfunctions. Male participants were purposely chosen to minimize potential hormonal influences on olfactory and gustatory functions, as sensory perceptions can markedly vary between genders and across different age groups. This controlled sample permits a more focused examination of the impact of dopamine deficiency on sensory function in RLS [17].

Moreover, the lack of random sampling could introduce selection bias, potentially affecting the accuracy of the observed associations between dopamine deficiency, olfactory dysfunction, and gustatory impairments. Unmeasured factors such as dietary habits, oral hygiene, and lifestyle choices may also confound the results, as these variables can independently influence both taste and smell perception apart from RLS. The cross-sectional design of the study further limits our ability to establish causality between dopamine deficiency and sensory dysfunctions in RLS.

The overlapping use of dopaminergic and gabapentin treatments among the participants further complicates interpretation, as these medications may independently influence sensory functions. Future research with a larger, more diverse cohort and employing a longitudinal approach, along with more optimum control of confounding factors, would help to resolve these limitations and offer a clearer understanding of the causal relationships.

Conclusion

This study signifies a notable association between RLS and sensory dysfunction, which suggests that simultaneous olfactory and gustatory deficits may indicate neurodegenerative processes in RLS. Future studies should include diverse populations and evaluate additional confounding variables, such as dietary habits, treatment types, and lifestyle factors. This knowledge could aid in creating targeted therapeutic approaches and help to establish sensory testing as a potential premature marker for neurodegenerative diseases in RLS patients.

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Effect on MDA-MB231 human breast cancer cell line treated with bilberry (*Vaccinium myrtillus*) using Annexin V and AgNOR staining

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Ethics Committee Approval
This study is a cell culture study that is outside the scope of studies requiring ethical approval.

Conflict of Interest
No conflict of interest was declared by the authors.

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Abstract

Background/Aim: Cancer has become a prevalent disease, emerging as one of the major chronic health issues today. Currently, common treatments against cancer include chemotherapy, radiotherapy, surgery, and the use of chemically synthesized drugs. However, despite significant advancements in diagnostic methods and treatments, drug resistance and metastasis remain primary hurdles to successful cancer therapy. Consequently, attention has been shifted towards exploring alternative treatments and therapies against cancer. This study sought to examine the time and dose-dependent effects of blueberry (*Vaccinium myrtillus* L) on MDA-MB-231 cell lines.

Methods: The study used the MDA-MB231 breast cancer cell line. We established three groups: control, 40 µl/ml bilberry, and 80 µl/ml bilberry, which were incubated at 37°C and 5% CO_2 for 24 and 48 h, respectively. After incubation, we examined the viability, apoptosis, and cell cycle of MDA-MB-231 cells with the Muse Cell Analyzer and assessed the status of nucleolar organizer region (NOR) proteins via silver nitrate (AgNOR) staining.

Results: Bilberry extracts were found to enhance apoptosis and exhibit a cytotoxic effect, thereby reducing cell proliferation in MDA-MB-231 cells after 24 and 48 h of culture. There was notably increased apoptosis at concentrations of 40 µl and 80 µl. Moreover, after 48 h of incubation, a significant difference emerged between the control and 40 µg/ml bilberry samples, notably in the average AgNOR count and the total AgNOR area/total nuclear area ratio.

Conclusion: Our study suggests that blueberries may be a potential therapeutic candidate for cancer treatment, thereby potentially enriching cancer research.

Keywords: MDA-MB-231, bilberry, apoptosis, *in vitro*

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Introduction

Despite significant advancements in cancer diagnosis and treatment, drug resistance and metastasis remain primary challenges. Current cancer treatments include chemotherapy, radiotherapy, and chemical drugs [1]. Although these treatments form traditional therapy procedures, plant-derived compounds account for a significant portion of today's pharmaceuticals, owing to their recognized medical importance in oncology [2]. Consequently, alternate cancer treatments and therapies are gaining prominence.

Epidemiological studies indicate lower cancer incidence rates within populations consuming high volumes of plant-based foods. Herbal medicines have been primary treatment sources for several years and remain in employment [3]. Research into the development of plant-based anticancer agents has surged since the 1950s [4]. Interestingly, around 75% of present-day anticancer agents are plant-derived [5].

Ingestion of fruits and vegetables rich in bioactive phytochemicals contributes notably to reducing the prevalence of cancer and other chronic diseases [6]. Research suggests that the anticancer properties of plants can be attributed to the phenolic compounds they contain. Both monophenolic and polyphenolic compounds in plant foods have demonstrated the potential to suppress or mitigate the onset, growth, and spread of cancer, both *in vivo* and *in vitro* [7].

The fruit and leaves of bilberries (*Vaccinium myrtillus*: VM) are abundant in phenolic compounds, including flavonoids [8]. Babova found that bilberry fruit houses five of the six natural anthocyanins also found in other fruits [9]. Seeram [10] identified bioactive food components, specifically in berry fruits, that showed potential in cancer prevention, as demonstrated by various preclinical studies.

Berries such as blackberries, blueberries, and strawberries, as well as their bioactive components, have been studied for their anticancer activities [11]. While most preclinical research centered on cancer prevention using berries targeted specific cancers like the esophagus [12-13], colon [14], lung [15], and skin [16], some studies found no significant benefits. That being said, blueberries (from VM), which are abundant in phenolic compounds and high in antioxidants, have shown promise in protecting against chronic diseases. These include cardio and cerebrovascular diseases, atherosclerosis, diabetes, and even cancer. Findings suggest that the active components in blueberries might work as effective anticancer agents, both as functional foods and nutritional supplements [17-18]. This study aims to explore the potential effects of VM treatment on NOR protein synthesis and its potential apoptotic effect on the MDA-MB-231 cell line.

Materials and methods

Preparation of *Vaccinium myrtillus* extract

We obtained the blueberries for this study from Artvin province in July, coinciding with their harvest time. We extracted juice using a juicer and transferred it to 50ml centrifuge tubes. Upon centrifuging the juice at 5500/min at 15°C for 30 min with a Selecta machine from Spain, we passed it through 0.45-µm and then 0.22-µm filters. We then froze the filtered juice into 7ml units, which we dried with a lyophilizer (Christ Freeze Dryer-Alpha 1-2 LD) to form a powder. We stored the bilberry extracts at -20°C until usage.

Cell culture

The MDA-MB-231 cell line, obtained from the American Type Culture Collection (Manassas, VA, USA), was cultured in Dulbecco's modified Eagles medium (DMEM, Capricorn Scientific, CP21-4310). This included streptomycin/penicillin (100 U/ml; Sigma Life Science, 046M4846V) and 15% fetal calf serum (FCS, Biowest, S181G-500) in a humidified 5% CO_2 atmosphere at 37°C (Sanyo, MCO-19 A/C(UV)). Healthy MDA-MB-231 cells were then divided into groups and subjected to a broccoli treatment. A tissue culture plate with 24 wells, each containing 1000 µL of medium and 1×10^5 MDA-MB-231 cells, was then utilized to determine the optimal VM dose. The cells were rinsed three times in 500 µl phosphate-buffered saline (PBS) after the medium was removed. Finally, experimental groups were formed by setting log concentrations of VM (40 and 80 µl/ml VM) on the breast cancer cells.

Cell viability assay and proliferation

We used the Trypan Blue cell counting method to determine the number of cells in each ml of the cell suspension. A portion of this cell suspension was mixed with an equal proportion of Trypan Blue solution in an Eppendorf tube. After a 5-min incubation period, the coverslip was shifted to both sides of the sealed Thoma slide (Marienfeld-Superior). We then differentiated and counted the stained and unstained cells under a microscope (Nikon Eclipse TS100).

Experimental design

For cell cycle testing, groups were created for 24 and 48-h incubations using Annexin V, along with 40 and 80 µl/ml VM control groups.

Annexin V assay

We conducted an apoptosis analysis using the Muse Cell Analyzer device alongside the compatible Muse Annexin V kit and dead cell assay reagent (Millipore; MCH100115). MDA-MB-231 cells were grown in 24-well plates, each containing 1x105 cells, and incubated for 24 and 48 h. Afterward, the cells were treated with trypsin and stained using Annexin V and a dead cell reagent, as per the manufacturer's guidelines. The stained cells were then analyzed with the Muse Cell Analyzer (Millipore Corporation).

Cell cycle assay

The MuseR Cell Cycle Kit (Millipore; MCH100106) was utilized to identify the cell cycle stage of MDA-MB-231 cells. These cells were grown in 24-well plates, with 1×10^6 cells per well, and were incubated for 24 and 48 h. Following this, the cells were treated with trypsin for removal. Subsequently, they were stained using the MuseR Cell Cycle Kit, per the manufacturer's protocols (Millipore Corporation), and analyzed using the Muse Cell Analyzer (Millipore Corporation).

AgNOR staining

MDA-MB-231 cells were cultured with a control, 40 µg/ml, and 80 µg/ml VM. They were then spread on a clean slide and allowed to dry at room temperature. After air drying, the slides were treated with fixative (a mixture of 3 volumes of methyl alcohol and 1 volume of acetic acid). These slides, once stained with silver nitrate (AgNOR), were examined and photographed

using a Leica DM 3000 light microscope and a Canadian-made Imaging Color 12 BIT digital camera. The images of MDA-MB-231 cells were transferred to ImageJ version 1.47 image processing software (National Institutes of Health, Bethesda, Maryland, USA) for further study. The total AgNOR area and the average AgNOR number per nuclear area were calculated by examining cell nuclei with the "freehand selections" tool.

Statistical analysis

The study evaluated the normal distribution using Shapiro-Wilk's test statistic, a histogram, and a q-q graph. Groups were compared using a one-way analysis of variance (ANOVA). Variance homogeneity was assessed using Levene's test. The differences between 24-h and 48-h measurements in each group were analyzed using a paired t-test. Tukey's test facilitated multiple comparisons. Per Tukey's test, if the alphabetic superscripts contain the same letter, it indicates no significant difference between the groups. Conversely, a difference implies statistical significance. Data were reported as means and standard deviations. Analysis was performed using TURCOSA statistical software (Turcosa Analytics Ltd Co, Turkey, www.turcosa.com.tr), with a significance level set at $P<0.05$.

Results

Annexin V findings

A significant difference was observed in the number of viable cells between the 24-h and 48-h measurements in the control group, as well as the 40 µl and 80 µl VM extract groups ($P<0.001$) (Figure 1). After 24 h of culture, both the 40 µl and 80 µl VM extract groups displayed significantly more viable cells than the control group ($P<0.001$). This trend continued after 48 h, with both VM extract groups maintaining significantly more viable cells than the control ($P<0.001$). However, there was no significant difference observed between the 40 µl and 80 µl VM extract groups (Table 1).

Figure 1: Annexin V test results.

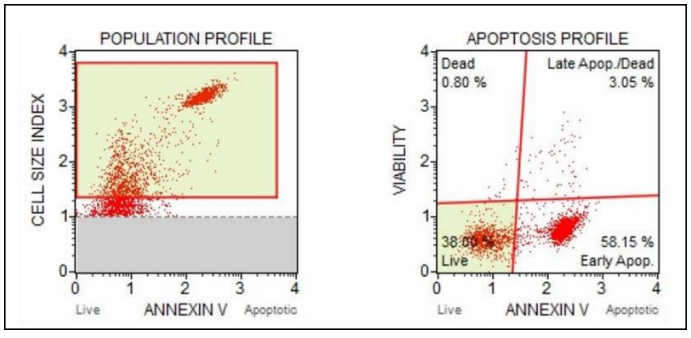

Table 1: In the Annexin V & Dead and live cells after 24 and 48 h.

Groups	Live Cell 24 h Mean (SD)	Live Cell 48 h Mean (SD)	*P*-value *
Control	98.62(0.63)a	84.81(0.39)b	<0.001
40 µl 40 µl VM	85.3(2.30)a	39.4(1.3)b	0.002
80 µl 80 µl VM	85.20(2.1)a	38.0(1.7)b	<0.001
***P*-value #**	<0.001	<0.001	

SD: standard deviation, *P**: One-way analysis of variance, *P*#: Paired t-test, VM: *Vaccinium myrtillus* L.

Cell cycle findings

G0/G1 findings: Upon evaluation of the G0/G1 measurement, a significant difference between all groups were identified based on both the 24 and 48-h results ($P<0.001$). The cell proportions in the G0/G1 stage were notably higher in all other groups, specifically the 40 µl VM extract and 80 µl bilberry groups, as compared to the control group ($P<0.001$). A significant difference was also identified between the 40 and 80 µl/ml VM extract doses when the 24 and 48-h results were considered. The 80 µl/ml VM extract was significantly higher compared to the 40 µl/ml VM group ($P<0.001$) (Table 2).

Table 2: G0/G1 measurement after 24 and 48 h in cell cycle test.

Groups	G0/G1 24 h Mean (SD)	G0/G1 48 h Mean (SD)	*P*-value *
Control	18.2(0.30)a	76.00(1.10)a	<0.001
40 µl 40 µl VM	21.6(0.4)b	91.2(0.3)b	<0.001
80 µl 80 µl VM	25.9(1.2)c	94.1(0.8)c	<0.001
***P*-value #**	<0.001	<0.001	

SD: standard deviation, *P**: One-way analysis of variance, *P*#: Paired t-test, VM: *Vaccinium myrtillus* L.

S measurement: A significant difference was observed between the 24-h and 48-h S measurements in the control group and the 40 and 80 µl/ml VM extract groups ($P<0.001$). The S measurement was higher than that of the control group, as well as the 40 and 80 µl/ml VM extract groups at both 24 and 48 h ($P<0.001$). A comparison of the 40 and 80 µl/ml VM groups revealed that the 80 µl/ml group recorded a lower measurement than the 40 µl/ml group ($P<0.001$). After 48 h, both the 40 and 80 µl/ml VM groups showed a significant reduction compared to the control group, with the decrease being more pronounced in the 80 µl/ml VM group ($P<0.001$) (Table 3).

Table 3: S measurement after 24 and 48 h in the cell cycle test.

Groups	S 24 h Mean (SD)	S 48 h Mean (SD)	*P*-value *
Control	44.90(0.2)a	12.3(0.1)b	<0.001
40 µl 40 µl VM	41.3(0.4)a	3.3(0.7)b	<0.001
80 µl 80 µl VM	39.4(0.3)a	1.8(0.2)b	<0.001
***P*-value #**	<0.001	<0.001	

SD: standard deviation, *P**: One-way analysis of variance, *P*#: Paired t-test, VM: *Vaccinium myrtillus* L.

G2/M measurement: Upon evaluating the G2/M measurement, a substantial decrease was noted in the 80 µl/ml VM group in comparison to both the control and 40 µl/ml VM groups after a 24-h culture period ($P<0.001$). A further statistically significant decline was seen in both the 40 and 80 µl/ml VM groups compared to the control after 48 h of culturing ($P<0.001$) (Table 4).

Table 4: G2/M measurement after 24 and 48 h in cell cycle test.

Groups	G2/M 24 h Mean (SD)	G2/M 48 h Mean (SD)	*P*-value *
Control	22.2(0.4)a	6.0(0.3)a	<0.001
40 µl 40 µl VM	22.5(0.9)a	2.4(0.2)b	<0.001
80 µl 80 µl VM	19.2(0.4)a	2.8(0.5)b	<0.001
***P*-value #**	<0.001	<0.001	

SD: standard deviation, P*: One-way analysis of variance, P#:Paired t-test, VM: *Vaccinium myrtillus* L.

AgNOR staining results: After 24 h of incubation, all VM groups showed no significant differences compared to the control group. However, after 48 h, there was a significant reduction in the mean AgNOR number in the 40 µg/ml VM group compared to the control group ($P<0.001$) (Table 5).

Table 5: Mean AgNOR number after 24 and 48 h of incubation.

H/Groups	Control	40 µg/ml VM	80 µg/ml VM	*P*-value
24 h Mean (SD)	3.78(1.09)	3.90(1.24)	3.96(1.19)	>0.05
48 h Mean (SD)	4.04(1.23)a	3.42(1.25)b	3.70(1.16)ab	<0.001

SD: standard deviation, AgNOR: Argyrophilic nucleolar organizer region, VM: *Vaccinium myrtillus* L.

Upon examining the 24-h incubation data, the TAA/NA value was not statistically significant. However, for the 48-h incubation data, this value significantly decreased ($P<0.001$) in the 40 µg/ml VM groups compared to the control group (Table 6).

Table 6: TAA/NA value at the end of 24 and 48 h of incubation.

H/Groups	Control	40 µg/ml VM	80 µg/ml VM	*P*-value
24 h Mean (SD)	0.05(0.03)	0.06(0.02)	0.06(0.02)	>0.05
48 h Mean (SD)	$0.08(0.03)^{a}$	$0.06(0.02)^{b}$	$0.07(0.03)^{ab}$	<0.001

SD: standard deviation, TAA/NA: Total AgNOR area (TAA)/Total nuclear area (NA) ratio, VM: *Vaccinium myrtillus* L.

Discussion

VM holds special significance among fresh fruits and vegetables due to its abundant antioxidants, anthocyanin levels, and other phenolic compounds [19]. It also provides a rich source of phenolic compounds like chlorogenic acid, quercetin, campherol, myricetin, procyanidin, catechin, epicatechin, and resveratrol. These compounds contribute to VM's antioxidant activity, and they are also rich in vitamin C [20].

Many studies have demonstrated the effects of VM, showing its anticancer properties, antioxidative potential, and antimicrobial and anti-inflammatory activities. There is also evidence to suggest its potential application in treating various diseases through clinical studies. VM appears to play a significant role in mitigating certain types of cancers, eye diseases, neurological disorders, and diabetes. Many of these studies opted to use extracted anthocyanins as opposed to direct VM juice. As such, anthocyanins are seen to contribute significantly to the VM plant's efficacy [21-23].

There's substantial evidence that VM hinders cancer's growth and spread. Studies suggest one way VM influences cancer is by prompting apoptosis in cancer cells, thereby inhibiting their growth. Zhao et al. [24] researched the potential anticancer effects of VM extracts loaded with anthocyanin against colon cancer. Their findings indicated that these extracts suppressed the growth of HT-29 cancer cells, demonstrating antitumor effects. In a 2008 study, Aiyer et al. [25] demonstrated that mice orally administered with whole VM powder experienced a 40% reduction in mammary tumor volume compared to the control group animals. Earlier studies corroborate that a diet rich in fruits and vegetables is linked with a decreased cancer risk [26].

Research findings support the antitumor effects of VM, warranting further investigation. Faria et al. [27] tested an anthocyanin bilberry extract and an anthocyanin-pyruvic acid adjunct extract on MDA-MB-231 and MCF7 cancer cell lines. Both extracts significantly reduced cell proliferation at 250 µg/mL after 24 h of incubation, with the adjunct extract manifesting a stronger effect on MDA-MB-231 cells. This suggests an estrogen receptor-independent activity. These findings confirm the efficacy of VM anthocyanins and related anthocyanin-pyruvic acid adducts as anticancer agents, inhibiting cancer cell proliferation [27].

Our findings align with these previous studies. We concluded that DNA synthesis was significantly inhibited after 48 h of treatment with 80 µl/ml VM extract. We observed that both 40 and 80 µl/ml VM extract doses slowed or stopped cell mitosis within 48 h in MDA-MB-231 cells.

Adams et al. [28] found that MDA-MB-231 human breast cancer cells treated with VM extract (12.5–25 µL/mL) for 72 h exhibited 1.5 times more cell apoptosis than the control group. In our study, we noticed an increased apoptotic response in cells treated with 40 and 80 µl VM extracts over 24 and 48 h.

The cytotoxic effects of this VM extract on MDA-MB-231 cells were evaluated in our study, and the doses were determined based on a pilot study [28].

Our study concluded that the application of 40 µl and 80 µl of bilberry extracts heightened apoptosis, exerted a cytotoxic effect, and hindered cell proliferation in MDA-MB-231 cells after 24 and 48 h of culture. Correspondingly, apoptosis increased in the 40 and 80 µl/ml VM groups after the same duration. A considerable decrease in viable cells was observed in both VM groups compared to the control group, showcasing the cytotoxic impact of bilberry on MDA-MB-231 cells.

Analysis of G0/G1 measurement results revealed minimal activity in the VM groups at the end of 24 and 48 h. Based on S phase data, DNA synthesis significantly diminished or halted in the 80 µl/ml VM group after 48 h. Evaluation of the G2/M phase results showed no evident effectiveness of the 40 µl/ml VM group after 24-h culture. Conversely, the 80µl/ml VM group showed a slowdown in mitotic division. Notably, a significant drop was recorded in the 40 and 80 µl/ml VM groups compared to the control group after 48 h. Hence, both doses - 40 and 80 µl/ml VM - effectively suppressed or halted mitosis.

Limitations

A limitation of this study is that it cannot analyze the anticarcinogenic properties of active substances in blueberries using cancer cell culture.

Conclusions

Our research found that VM augments apoptosis in MDA-MB-231 human breast cancer cells by various mechanisms, consistent with other studies on VM. We also found that the AgNOR number increases after 48 h of incubation at a 40 µl/ml VM dose. These findings suggest the potential use of AgNOR as a biomarker for determining the therapeutic dosage and highlight VM as a potential candidate for use against cancer. Additionally, incorporating blueberries into dietary cancer prevention strategies may be beneficial. Future *in vitro* studies and further investigations into how blueberries modulate the metastatic potential of MDA-MB-231 cells, currently being studied in vivo, will enrich the related literature.

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Case report
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A case of chronic exocrine pancreatic insufficiency in a gastric bypass patient

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Informed Consent
The authors stated that the written consent was obtained from the patient presented with images in the study.

Conflict of Interest
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Abstract

Exocrine pancreatic insufficiency (EPI) is a significant complication of bariatric surgery, but its frequency and outcomes are not well-studied. If EPI is not diagnosed, it can result in nutritional deficiencies, dehydration, and acute kidney damage. Our patient, a 47-year-old woman, underwent Roux-en-Y gastric bypass (RYGB) surgery due to morbid obesity four years ago and lost contact with outpatient follow-ups. She came to us presenting chronic diarrhea and fatigue and was diagnosed with chronic pancreatic exocrine deficiency based on a low fecal elastase level. After starting her on pancreatic enzymes, her symptoms resolved. It is crucial to maintain a high degree of suspicion in order to diagnose EPI in patients who have undergone RYGB. The fecal elastase test, which is both reliable and inexpensive, is an effective diagnostic tool for EPI; prompt treatment can alleviate symptoms within days.

Keywords: exocrine pancreatic insufficiency, Roux-en-Y gastric bypass, fecal elastase, pancreatic enzyme replacement therapy

Introduction

Exocrine pancreatic insufficiency (EPI) is a common complication following Roux-en-Y gastric bypass (RYGB) surgery. Diagnosis can be confirmed via a positive fecal elastase-1 test or noticeable improvement following pancreatic enzyme replacement therapy [1]. Fecal elastase is a highly accurate diagnostic test for EPI [2]. Post-operative diarrhea is a common issue after this type of surgery, with potential causes including dumping syndrome, short bowel syndrome, small intestinal bacterial overgrowth (SIBO), malabsorption of bile acids or carbohydrates, and EPI.

EPI, which can result from RYGB due to irregular secretion of pancreatic enzymes, features additional symptoms such as fat maldigestion and steatorrhea. These occur due to procedure-related changes [3]. Therefore, EPI should always be considered as a potential cause of post-operative diarrhea following RYGB surgery.

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Case presentation

A 47-year-old female patient who previously underwent RYGB surgery for morbid obesity 4 years prior presented with complaints of loose stool, fatigue, headache, and nausea that had persisted for 2 weeks. She had not been regularly followed up on an outpatient basis since her surgery. Daily, she experienced six to eight episodes of greasy diarrhea, which worsened after consuming fatty meals. The patient denied any unintentional weight loss or instances of melena. She reported fatigue and progressive weakness, becoming tired even with minimal activity. Her appetite had significantly declined since the onset of these symptoms, and nausea limited her ability to eat and drink.

During the presentation, her vital signs were recorded as follows: temperature 35.5°C, pulse 84 beats per minute, respiratory rate 18 breaths per minute, and blood pressure 122/71 mmHg with a mean arterial pressure of 99. The physical examination was largely normal, the exception being conjunctival pallor and lower abdominal tenderness; however, there was no abdominal rigidity or guarding.

Her complete blood count (CBC) was mostly normal, apart from normocytic anemia, with a hemoglobin level of 7.7 g/dl. The liver function tests showed regular results, with a slight increase in aspartate aminotransferase (AST). Her creatinine level was high (3.6 mg/dl, compared to a usual range of 1.5–1.7 mg/dl). The bicarbonate was 7 mEq/L at admission, and the anion gap was 9. Venous blood gas (VBG) displayed a pH of 7.05 and a $paCO_2$ of 27.

Her iron studies were standard, except for a mild decrease in serum iron to 38 µg/dl. The total iron binding capacity was 272 µg/dl, with transferrin saturation at 14% and ferritin at 15 ng/ml. Vitamin B12 and folate levels were standard at 598 pg/mL and 13.7 ng/mL, correspondingly. The thyroid stimulating hormone (TSH) was 1.811 mIU/L. However, her vitamin D level was critically low at 10 ng/mL.

The urinalysis indicated hazy urine, displaying positive results for both urine nitrite and leukocyte esterase, accompanied by an excess count of white blood cells (WBC) beyond measurable limits in the high-power field. The urine culture revealed pan-sensitive *E. coli*. A computed tomography (CT) scan of the chest, abdomen, and pelvis, performed without intravenous contrast, displayed no acute conditions. No abnormalities were spotted on the chest X-ray and renal ultrasound. There was an increase in stool fat, and the Fecal Pancreatic Elastase was recorded below 10 µg/g, a reduction from 23 µg/g during her admission 18 months previously. Evaluations for stool WBC, C difficile, stool ova, and parasites returned negative. Additionally, both stool and blood cultures showed no growth.

The esophagogastroduodenoscopy (EGD) revealed a gastric bypass with a small pouch and an intact staple line. It showed a gastrojejunal anastomosis with a healthy-looking mucosa and a standard esophagus. During the colonoscopy, the terminal ileum and colon appeared normal. Biopsies were taken from the right and left colon for histology. The colonic biopsy demonstrated an acute colitis pattern with preserved architecture of the colonic mucosa and several occurrences of cryptitis and crypt abscess. However, no features of chronicity, granulomatous inflammation, dysplasia, or malignancy were observed.

Given her lab results and medical history, she was initially diagnosed with acute kidney injury (AKI) superimposed on chronic kidney disease (CKD), normal anion gap metabolic acidosis, chronic diarrhea, urinary tract infection (UTI), and iron deficiency anemia.

The primary cause of the patient's symptoms was EPI, which led to diarrhea. This was verified by lab results, leading to the initiation of a pancrelipase (Creon) treatment. The patient was advised to eat small, frequent meals and to take pancreatic lipase with each meal and snack. A dosage of pancrelipase 24,000 Units was prescribed: two tablets three times a day with meals and one tablet two times a day with snacks. Following two days of this treatment, the patient noted a substantial improvement in their condition. There were no subsequent episodes of diarrhea, nausea, vomiting, or abdominal pain.

The presumed cause of AKI in CKD was chronic diarrhea, which led to acute tubular necrosis and acute interstitial nephritis. After proper hydration and cessation of her diarrhea and Naproxen use, her creatinine levels improved to 1.5 mg/dl.

The assumption was that the normal anion gap metabolic acidosis resulted from chronic diarrhea and renal tubular acidosis. Following sufficient diarrhea control and sodium bicarbonate treatment, the bicarbonate and VBG pH became normal at 19 mEq/l and 7.28, respectively. Outpatient sodium bicarbonate treatment was maintained.

She began taking ferrous sulfate for iron deficiency anemia and calcium carbonate and ergocalciferol for vitamin D deficiency. Multivitamins containing folic acid and thiamine mononitrate were also resumed as nutritional supplements following her gastric bypass.

After 5 days of inpatient therapy and four days of pancrelipase therapy, she was discharged. She was advised to follow up at an outpatient gastroenterology clinic. Her symptom resolution remained consistent after 6 months.

Discussion

Adults with a body mass index (BMI) of 35 kg/m^2 or higher are suggested to undergo bariatric surgery, whether they have obesity-related comorbidities or not. This procedure is also recommended for diabetic patients with a BMI of at least 30 kg/m^2. Bariatric surgery's benefits on weight loss, comorbidity improvement, cancer risk reduction, and long-term mortality are well-documented [4]. However, this surgery can cause complications like postprandial hypoglycemia, abdominal pain, anastomotic stenosis, deficiencies of iron, vitamins B12, folic acid and D, calcium deficiency, loss of bone density, and kidney stones [5]. Moreover, EPI is a common complication occurring in up to 41.6% of patients after such a procedure and is more prevalent in RYGB compared to sleeve gastrectomy [1].

In the given case, a patient who underwent RYGB 4 years ago due to morbid obesity (BMI ≥40 kg/m^2) started showing signs of potential EPI, like diarrhea and steatorrhea, for 2 weeks. This prolonged diarrhea led to an AKI and normal anion gap metabolic acidosis. Iron deficiency and vitamin D deficiency can occur in up to 49% of patients who have undergone RYGB [6,7]. Our patient, who stopped taking her nutritional supplements, also showed deficiencies in iron and vitamin D levels.

Fecal elastase concentration testing is a cost-effective and straightforward method for diagnosing moderate to severe EPI. The patient's low levels of fecal elastase and significant improvement with pancreatic enzyme replacement therapy confirmed the EPI diagnosis. Prompt treatment with pancreatic enzyme replacement therapy led to a swift resolution of symptoms.

Conclusion

EPI is a key complication of RYGB surgery. This condition may cause symptoms such as diarrhea and steatorrhea. If post-operative patients experience diarrhea, a high suspicion of this condition is necessary. Fecal elastase, an affordable and reliable diagnostic test, is used to confirm EPI. Based on the clinical symptoms and/or low fecal elastase levels, pancreatic enzyme replacement therapy can be initiated to alleviate symptoms.

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Case report
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Chest pain during a daily run unfolds an unfortunate diagnosis: A case report and review of the literature on spontaneous coronary artery dissection

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Abstract

Spontaneous coronary artery dissection (SCAD) is an uncommon but important cause of acute coronary syndrome. This condition is not linked to typical atherosclerotic risk factors, such as tobacco use, high blood pressure, diabetes, and hyperlipidemia. The majority of patients with SCAD experience chest pain and have some form of acute coronary syndrome with acute ST-elevation myocardial infarction (STEMI) being the most common. Early diagnosis is of utmost importance, as it can be fatal if management is delayed. Medical management is preferred over percutaneous intervention for hemodynamically stable cases. A case is presented of a 51-year-old female patient with no significant comorbidities who presented with exertional chest pain and STEMI, which unfolded a diagnosis of SCAD.

Keywords: chest pain, SCAD, spontaneous coronary artery dissection, coronary angiogram

Introduction

Spontaneous coronary artery dissection (SCAD) is defined as a dissection/tear of a coronary artery that is not related to atherosclerosis, trauma, or iatrogenic causes [1,2]. It is a rare cause of acute coronary syndrome (ACS) and commonly occurs in young healthy women aged 40-50 [3,4]. In SCAD, ACS is caused by blockages in the coronary arteries resulting from either intramural hematoma or intimal disruption, as opposed to atherosclerotic plaque [5]. Due to the frequent utilization of coronary angiography, there has been increased recognition of SCAD in patients presenting with ACS, with an overall incidence ranging from 0.28% to 1.1% [6]. Patients with SCAD experience chest pain and present with some form of ACS, with STEMI being the most common presentation. Coronary angiography is often the diagnostic modality used for SCAD, although intravascular ultrasound or coronary tomography has been used in rare conditions [5]. Management of SCAD is controversial and depends on the patient's characteristics and presentation. Most of the time, SCAD is managed medically without immediate revascularization. Nevertheless, percutaneous intervention may be required if the patient is hemodynamically unstable or has recurrent chest pain.

We report a case of a middle-aged healthy woman who presented with acute ST-segment elevation myocardial infarction (STEMI) and was found to have SCAD.

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Case presentation

A 51-year-old Caucasian healthy female presented to the emergency department with exertional substernal chest pain. She reported that the pain started when she was jogging. She described the experience as a throbbing pain in her mid-chest, non-radiating, and rated it ten out of ten in intensity, which slightly improved with rest. It is associated with lightheadedness and dizziness. She denied any chest pain before the episode. There was no reported history of hormonal contraceptive use, diabetes mellitus, hypertension, dyslipidemia, smoking, or hypothyroidism. Both parents had coronary artery disease at the age of 60 but there was no family history of SCAD, fibromuscular dysplasia (FMD), autoimmune disease, or malignancy.

The patient experienced moderate distress owing to chest pain. Her blood pressure was 140/90 mmHg in the right and 136/86 mmHg in the left arm. She had a normal lung and cardiac examination. An initial electrocardiogram (ECG) showed ST-segment elevation in the inferior leads, as shown in Figure 1. The initial troponin T level was 0.17 ng/ml (normal <0.03 ng/ml). The patient was quickly administered a loading dose of aspirin 325 mg, ticagrelor 180 mg, heparin 4000 U, and sublingual nitrate. Given the evidence of inferior wall STEMI and refractory chest pain, emergent coronary angiography was done, which revealed SCAD with 99% luminal stenosis in the mid-right coronary artery (RCA), as shown in Figure 2, with normal flow in the left anterior descending and left circumflex arteries. The patient had a successful stent placement on her mid-RCA with a resumption of TIMI-3 flow (Thrombolysis in Myocardial Infarction-3) (Figure 3). There was no ST-segment elevation on the EKG that was performed after cardiac catheterization (Figure 4).

The complete blood count, complete metabolic panel (CMP), lipid panel, thyroid function test, and glycated hemoglobin (HbA1C) levels were unremarkable. The urine pregnancy test result was negative. The laboratory work is shown in Table 1. Transthoracic echocardiography (TTE) revealed a left ventricular ejection fraction of 60% with normal left ventricular wall motion and normal valves. Computed tomography angiography (CTA) of the head and neck revealed subtle areas of bead appearance in the mid to distal cervical internal carotid artery, suggestive of FMD (Figure 5). Abdomen/Pelvis CTA showed superior left main renal artery with possible slight beading of the vessel, which was suspicious for FMD without frank aneurysm or stenosis. No other abnormalities were observed in the visceral vessels. Serologic tests were also performed to rule out secondary causes of SCAD. These included tests for rheumatoid factors, cyclic citrullinated C peptide antibody, antinuclear antibody screening, anti-double-stranded DNA antibody, and U1-ribonucleoprotein antibody, which were all negative.

The patient was discharged with aspirin, ticagrelor, atorvastatin, metoprolol succinate, and nitroglycerin. It was recommended that she follow up with a primary care physician, cardiologist, and cardiac rehabilitation.

After hospitalization, the patient had one admission and two emergency visits within the next two months. She presented with chest tightness associated with shortness of breath and palpitations. EKG and troponin levels were unremarkable during each visit. A cardiology team was consulted during each visit. Her symptoms resolved after extensive counseling and treatment for her anxiety. The patient has been doing well for the last six months and has regularly followed up with cardiology examinations.

Figure 1: ECG on admission showing ST-segment elevations in lead II, III, and aVF.

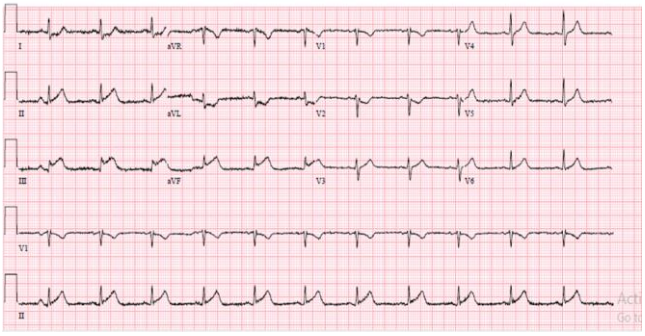

Figure 2: Coronary angiogram showing SCAD in mid-RCA

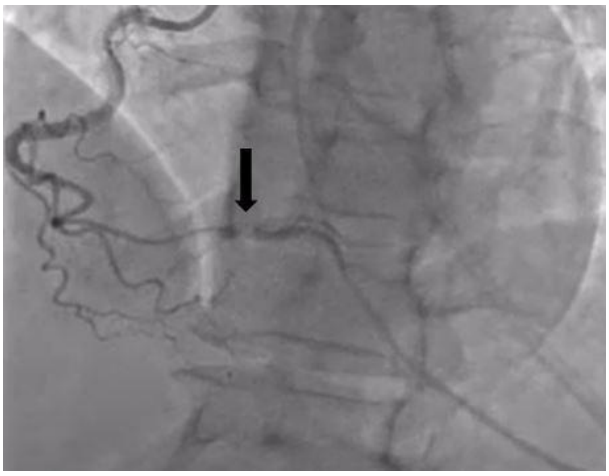

Figure 3: Coronary angiogram showing normal flow in the stenotic vessels after placement of stent

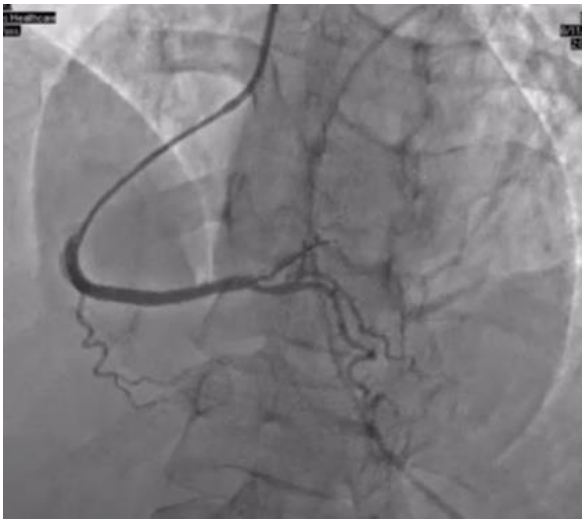

Figure 4: Repeat EKG after successful stent placement in mid-RCA showed no more ST-segment elevations in inferior leads.

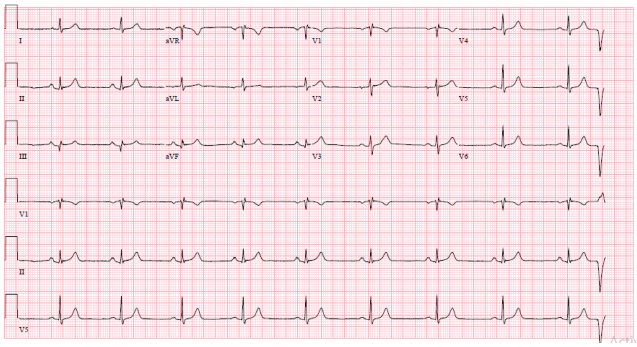

Figure 5: CTA of head and neck showing subtle areas of bead appearance of mid to distal cervical ICA.

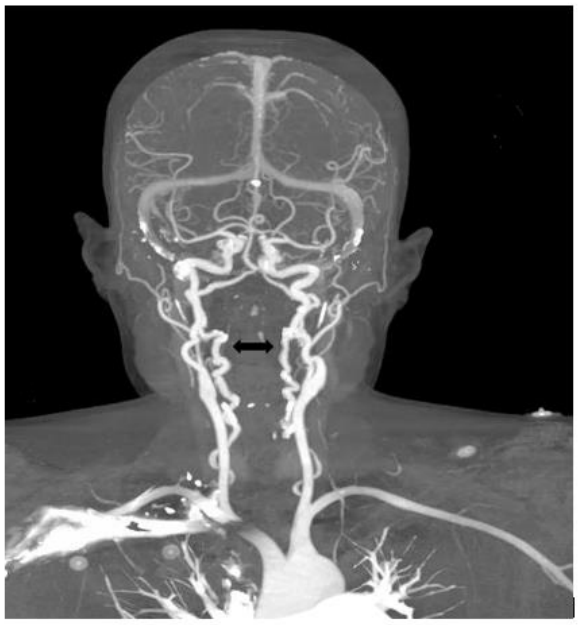

Table 1: Lab values along with their reference range

Labs	Values	Normal references values
WBC	5.6 K/µL	4-11 K/µL
Hemoglobin	12.5 g/dl	12-15 g/dl
Platelets	169 K/µL	150-400 K/µL
Sodium	140 mEq/L	135-145 mEq/L
Potassium	3.9 mEq/L	3.5-5 mEq/L
Blood Urea Nitrogen	16 mg/dl	8-21 mg/dl
Creatinine	0.9 mg/dl	0.6-1.2 mg/dl
HbA1C	4.7%	3.6-5.6 %
LDL	103 mg/dl	<100 mg/dl
HDL	66 mg/dl	>50 mg/dl
Total Cholesterol	179 mg/dl	<200 mg/dl
Triglycerides	31 mg/dl	<150 mg/dl

K/µL: thousand/microliter, g/dl: gram/deciliter, mEq/L: milliequivalent/liter, mg/dl: milligram/deciliter

Discussion

The coronary arteries, which supply blood to the heart muscles, have three layers: tunica intima, tunica media, and tunica adventitia [7]. Coronary artery dissection occurs when there is a separation between any of these adjacent layers. The exact etiopathogenesis of SCAD is still not fully understood. Further research is needed to definitively determine the primary initiating event in the development of SCAD. Two hypotheses have explained the pathophysiological process of coronary artery dissection. According to the “inside-out” hypothesis, an endothelial intimal disruption or "flap" allows blood to enter the subintimal space from the true lumen. This leads to the formation of an intramural hematoma that dissects the arterial wall layers. In the “outside-in” hypothesis, the hematoma arises *de novo* in the media, possibly from disruption of traversing microvessels within the arterial wall. This then leads to dissection of the layers of the arterial wall [8].

Coronary artery dissection can result from chest trauma, coronary angiography, or extension of aortic dissection. SCAD is the dissection of the coronary arteries without any obvious etiology, as listed above [1,2]. SCAD leads to the blockage of the coronary artery due to the narrowing of its lumen, which can be caused by either a dissection flap or the spread of an intramural hematoma [9]. SCAD was first reported in 1931 in the autopsy of a 42-year-old woman [10]. According to angiographic evaluations, the incidence of SCAD is approximated to be between 0.28% and 1.1%. [6]. Literature review shows that almost 90% of patients with SCAD are young, healthy females between the ages of 47 and 53 [11].

SCAD has been observed to occur before or during a woman's menstrual cycle. This has been reported in women who are using hormonal contraceptives, postmenopausal hormone therapy, or receiving infertility treatment due to a history of infertility. In addition, pregnancy-associated SCAD (P-SCAD) can occur at any stage during pregnancy or up to 12 weeks postpartum, but the majority of cases occur in the early postpartum, usually in the first week [8].

SCAD has been associated with triggers, such as emotional and physical stress including the Valsalva maneuver, vomiting, retching, and pregnancy [12]. Several instances have been recorded in case reports linking SCAD with inflammatory and autoimmune conditions, such as systemic lupus erythematosus, inflammatory bowel disease, sarcoidosis, and celiac disease [3,4,12-14]. Many studies have also reported an association of SCAD with fibromuscular dysplasia. It has been hypothesized that SCAD might be an initial manifestation of fibromuscular dysplasia [14-16].

Approximately 90% of people presenting with SCAD have some form of ACS, of which 20 to 50% present with ST elevated myocardial infarction [4,17]. Other presentations include ventricular arrhythmias, cardiogenic shock, or sudden cardiac death [18,19]. The left anterior descending artery is the most commonly affected artery, which accounts for 32% to 46% of all cases, followed by the circumflex and the right coronary artery. [14,18,19]. Chest pain is the most common symptom of SCAD, which prompts the patient to visit the emergency room [20].

SCAD is typically diagnosed through coronary angiography. In some cases, additional imaging tests, including intravascular ultrasonography, optical cardiac tomography, or coronary computed tomographic angiography (CCTA) may be used to further delineate the SCAD lesion and visualize features like dissection flaps and intramural hematomas [8,21-23]. In a prospective study of 327 SCAD patients, research found that hypertension was more than two times likely to be associated with an increased risk of recurrent SCAD. Conversely, the use of beta-blocker medications was found to be significantly associated with a reduced risk of recurrent SCAD by almost two-thirds [14]. According to this study, the majority of patients (83.1%) received initial medical treatment, while only 16.5% or 2.2% underwent in-hospital percutaneous coronary intervention or coronary artery bypass graft surgery, respectively.

Early recognition is of key importance in the management of SCAD. The decision to attempt revascularization or medically treat acute myocardial infarction due to SCAD depends on the severity of the disease and various other factors. No randomized clinical trials have compared medical management with immediate revascularisation in SCAD. Medical management should be based on each individual, including the use of statins, beta-blockers, antihypertensive medications, and antiplatelet medications [10]. Medical management is generally preferred over percutaneous intervention/stent placement, mainly if the patient is hemodynamically stable [8,21]. The risk of hematoma propagation, iatrogenic injury, wire placement in the false lumen, and the need for multiple stents are some of the reasons why PCI is a challenging option in SCAD. Studies have

indicated that most SCAD lesions that are medically treated tend to heal with time. These lesions generally show an improvement in blood flow and a reduction in severity, which can be observed through angiography [18,22].

Research has also shown that SCAD survivors have significant rates of psychological distress, including anxiety, depression, and posttraumatic stress disorder [24]. Our patient presented to the hospital three times after her diagnosis of SCAD, including one admission and two ER visits, during which no abnormalities were found. Extensive psychological support was given, together with a referral to the SCAD survivors community group.

Conclusion

SCAD is often misdiagnosed and managed as atherosclerotic acute coronary syndrome (ACS), which can be catastrophic for the patient. Accurate and timely diagnosis is crucial, as it not only provides appropriate supportive care but also helps stratify patients and ensure that percutaneous coronary intervention (PCI) is performed selectively in appropriate patient groups with SCAD. This is important because PCI for SCAD is associated with higher rates of complications and a lower success rate in comparison to PCI for atherosclerotic coronary artery disease.

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