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PONCE HEALTH SCIENCES UNIVERSITY

SCIENTIFIC JOURNAL

REGIONAL DISPARITIES IN COVID-19 MORTALITY RATES: An Analysis of the 2021 National Inpatient Sample

HERMANSKY-PUDLAK SYNDROME: A Bilingual Assessment of Quality and Readability of Online Health Information

BRIDGING THE GAPS IN HEALTHCARE: The journey of a student-run free clinic in Puerto Rico

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EDITOR'S LETTER

We are proud to launch the inaugural edition of the peer-reviewed PHSU-Scientific Journal, a moment that represents both a celebration of academic achievement and a bold step forward in our university's commitment to research excellence. This first volume brings together manuscripts from a wide range of disciplines, each offering unique perspectives that converge under a shared mission to advance knowledge.

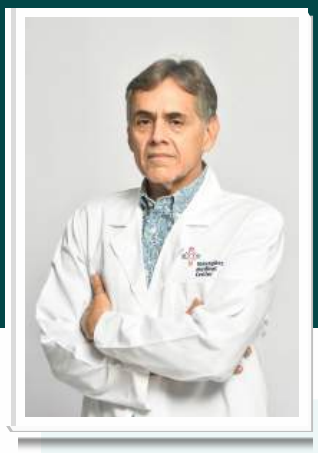
The idea behind this journal was simple yet powerful: to bring our university community closer to research. We aim to create a dynamic space where students, trainees, and faculty from all academic backgrounds feel inspired and empowered to explore, create, and lead through research. We believe that by fostering a spirit of curiosity and collaboration across disciplines, we can ignite new ideas, cultivate innovation, and drive scientific progress; not only within Puerto Rico but also on a global scale.

Through this publication, we seek to motivate the next generation of researchers to view research not just as a career path, but as a vital contribution to society. Each article in this edition reflects the creativity, rigor, and passion of our investigators, and serves as a testament to what can be achieved when diverse minds come together with a common purpose: to generate knowledge with impact.

As we celebrate this important beginning, I invite all members of the PHSU scientific community, along with collaborators from across institutions, to engage, contribute, and innovate with vision for the future. May this journal serve as a platform that continues to grow, inspire, and showcase the incredible research potential that exists right here in Puerto Rico, for the benefit of the world.

Thank you for joining us on this journey.

DR. WILFREDO DE JESUS
EDITOR-IN-CHIEF



EDITOR'S LETTER

Dear Readers,

It is with great excitement and anticipation that I welcome you to the inaugural edition of PHSU Scientific Journal, a new platform dedicated to advancing knowledge and fostering dialogue within the scientific community. Launching this journal represents the culmination of vision, collaboration, and dedication, and we are thrilled to embark on this journey with you.

At PHSU Scientific Journal, our mission is to serve as a catalyst for innovation, inquiry, and scholarly excellence. We aim to provide a platform that not only showcases groundbreaking research, but also encourages interactions between disciplines, paving the way for novel insights and transformative ideas. Our goal is to bridge gaps and foster collaborations that will address the pressing challenges and opportunities within our society.

This first edition includes a collection of high-quality articles that exemplify the diverse research and innovative thinking we seek to promote. Each article has undergone a rigorous peer-review process, ensuring the integrity and scientific accuracy of the work we present. We are proud to feature contributions from both established researchers and emerging voices in the field, reflecting the journal's commitment to spreading scientific knowledge.

We envision PHSU Scientific Journal as a dynamic and evolving entity, responsive to changes in the growing landscape of sciences. Our commitment to open dialogue and accessibility aims to inspire deeper engagement, broader participation, and more inclusive discourse.
We encourage contributions from researchers, practitioners, and thought leaders from around the globe. Your participation, whether as authors, reviewers, or readers, is vital to fulfilling the journal's vision.

We are grateful to our initiators, Laura and Karla, Fundación Intellectus, Editor in Chief, editorial board, reviewers, and contributors who have worked diligently to make this inaugural edition possible. Their expertise, passion, and commitment are the foundation upon which PHSU Scientific Journal is built.

As we are launching this new endeavor, we invite you to join us in this exciting venture. Your feedback, collaboration, and engagement will be crucial in shaping the future of the journal. We look forward to your contributions and to fostering meaningful discussions within our pages.
Thank you for your support and trust in PHSU Scientific Journal. Together, let us push the boundaries of knowledge and explore new frontiers.

DR. SIMON CARLO
MANAGING EDITOR

MEET
THE COFOUNDERS

KARLA SANTIAGO-SOLTERO, MD

LAURA SANTIAGO CAOBI, MD

As co-founders of the PHSU Scientific Journal, we, Karla Santiago-Soltero and Laura Santiago Caobi, are driven by a shared vision to elevate the voices of researchers and academics in Puerto Rico. We created the journal to provide a platform that not only showcases the exceptional work being conducted on the island but also amplifies its impact on the global stage.

Our mission is to foster a culture of academic excellence and collaboration by offering a peer-reviewed, open-access publication platform that adheres to the highest standards of scientific integrity. Through this initiative, we aim to bridge gaps in accessibility, support emerging scholars, and highlight the groundbreaking contributions of our scientific community. We are proud to contribute to the advancement of research and to empower the next generation of innovators in Puerto Rico.

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***For further details, please refer to the *Submissions* page at phsujournal.com!**

Scope & Mission

The PHSU Scientific Journal publishes original, peer-reviewed work from the Ponce Health Sciences University community, including the Schools of Medicine, Behavioral & Brain Sciences, Public Health, Nursing, Dentistry, and affiliated healthcare institutions. We aim to elevate scientific contributions from students, faculty, and professionals across Puerto Rico.

Formatting

Use Times New Roman 12 pt, double-spaced, 1" margins. Submit manuscripts in Word format using the official journal template. Include page numbers. Label and cite all tables/figures in order.

Accepted Article Types

Article Type	Title Length	Abstract (Words)	Word Count	Conclusion	References	Tables/Figures
Original Article	250 characters	150-250	Intro: 500-800 Methods: 700-1200 Results: 500-800 Discussion: 800-1500 Conclusion: 150-300	150-300 words	≥30	≤8
Review Article	250 characters	150-250	Intro: 800-1500 Methods: 5000-10000 Results: 800-1500 Discussion: 500-1000 Conclusion (*not mandatory*): 100-200	100-200 words	≥40	≤8
Case Report	250 characters	150-250	Intro: 300-700 Case presentation: 250-500 Discussion: 500-1000	-	15-40	≤2
Clinical Pearls	250 characters	-	Up to 1000	-	≤5	≤2
Innovative imaging	250 characters	-	Up to 1000	-	≤5	≤10
Research Letter	250 characters	150-250	Up to 1600	-	≤5	≤2
Letter to the Editor	250 characters	-	Up to 1200	-	≤5	-

Submission Procedure

Online Submission: Manuscripts must be submitted through the designated online portal on the journal's website (phsujournal.com).

Peer Review: This system is used to assess the quality of a manuscript before it is published, carried out by independent experts in the relevant research fields. The PHSU Scientific Journal is a single-blind peer review publication.

Deadlines: Submission deadlines will be established by the Board and will be notified via Call-to-Action bulletins sent through the institutional email.

Decision Timing: Upon manuscript acceptance, the Board will inform the corresponding author of their decision for publication within 4-6 weeks.

Resubmission: Once the manuscript with reviewer comments has been sent to the corresponding author, they will have 1-2 weeks to make any revisions/corrections suggested. Upon resubmission, the author will receive the Board's final decision within 4-6 weeks.

Copyright Statement

ORIGINAL ARTICLES

Original Article

Regional Disparities in COVID-19 Mortality Rates: An Analysis of the 2021 National Inpatient Sample

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Abstract: Amid the 2019 coronavirus (COVID-19) pandemic, regional disparities in outcomes were identified as crucial for an effective public health response. Regional variations in COVID-19 outcomes in the United States of America (USA) were studied in this retrospective cross-sectional analysis, with a focus on in-hospital mortality rates among COVID-19 patients with pneumonia or respiratory failure, using the 2021 National Inpatient Sample dataset. Patients aged ≥18 years with confirmed diagnoses of COVID-19 associated pneumonia or respiratory failure were included in the study. The association between hospital census regions and in-hospital mortality rates was examined using multivariate logistic regression, adjusting for demographic, clinical, and hospital characteristics. Significant disparities in regional COVID-19 outcomes in the USA were revealed by the results. Mortality rates in the COVID-19 study cohort ranged from 14% in the Midwest to 18% in the West. Adjusted significant differences were shown by regression analysis, with the West exhibiting up to 28% higher odds of death (odds ratio: 1.28, 95% confidence interval: 1.218–1.354, $p<0.001$) than the Midwest, the region with the lowest mortality. The importance of accounting for demographic, clinical, and contextual factors in understanding these disparities and addressing regional disparities to promote health equity during the ongoing pandemic was underscored by these substantial regional variations in COVID-19 mortality rates in the USA.

Keywords: COVID-19 pneumonia mortality rates; National Inpatient Sample; Regional disparities; Respiratory failure

1. Introduction

In the wake of the 2019 coronavirus (COVID-19) pandemic, a thorough analysis of outcomes across large populations is needed to enhance readiness for future health crises, with a particular focus on healthcare outcomes across diverse regional landscapes. In a recent study, Stoto et al. delineated the regional disparities in COVID-19 mortality within the United States of America (USA), illustrating a division into "two Americas," characterized by differing attitudes towards vaccination and public health measures [1] . Their examination of excess mortality rates highlighted geographic variations, with the south notably experiencing mortality rates consistently higher than the national average, while socioeconomic factors, such as poverty rates and income inequality, further exacerbated these disparities. Bollyky et al. emphasized the significance of analyzing regional disparities in COVID-19 outcomes and policy responses [2]. Through an observational analysis addressing critical policy-relevant questions, including socioeconomic and racial inequities, healthcare and public health capacity, and the impact of policy mandates on the balance between COVID-19 infections, deaths, and socioeconomic outcomes, they uncovered significant state-

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infections, deaths, and socioeconomic outcomes, they uncovered significant state-by-state variations in the standardized cumulative COVID-19 death rates. Factors such as lower poverty levels, higher educational attainment, and improved healthcare access were associated with lower infection and mortality rates. Enhanced health outcomes were linked to the enforcement of safety mandates, the use of masks, and elevated vaccination levels.

Moreover, historical studies during previous pandemics, such as the 1918 influenza pandemic and the H1N1 influenza pandemic, have also demonstrated stark disparities in mortality rates [3,4]. Research has shown that suboptimal geographic accessibility to comprehensive HIV care further exacerbates mortality disparities [5].

In this study, the regional disparities in COVID-19 outcomes were explored, particularly in-hospital mortality among COVID-19 patients with pneumonia or respiratory failure, within the healthcare framework of the USA, utilizing the extensive and publicly available 2021 National Inpatient Sample (NIS) database. Although the NIS has previously offered insights into the COVID-19 pandemic, its extensive 2021 dataset presents an unparalleled opportunity for novel perspectives, particularly given the increased testing availability, vaccine distribution, and emergence of new viral variants during that year [6-8]. This study fills crucial gaps in the existing literature, as unlike previous studies that predominantly explored demographic disparities, particularly those related to race and ethnicity, this study evaluated the impact of census-region-based differences on healthcare outcomes and examined the factors potentially contributing to these differences.

2. Methodology

A retrospective, cross-sectional analysis was conducted using data from the 2021 NIS database managed by the Agency for Healthcare Research and Quality. The NIS collects a 20% stratified sample of patient stays from 4,500 non-federal, short-term general and specialty community hospitals. This collection method employs specific weighting to ensure nationwide hospital representation and facilitates the extrapolation of consistent national estimates. In 2021, the NIS compiled data from 47 states and Washington, DC, in the USA.

Each record in the NIS corresponds to a hospitalization occurrence rather than a distinct patient [9]. To streamline the statistical interpretation and analysis in this study, each instance of hospitalization was considered as an individual patient encounter. This methodology can be implemented even in situations where patients may be admitted multiple times for similar clinical conditions.

The study cohort comprised individuals aged 18 years or older admitted to the hospital, all of whom had a discharge diagnosis of COVID pneumonia [International Classification of Diseases, Tenth Revision (ICD-10) code J12.82], indicating pneumonia caused by the novel coronavirus SARS-CoV-2, confirmed at the time of discharge. Additionally, patients with a confirmed diagnosis of COVID-19 (ICD-10 code U07.1) plus concurrent diagnoses of acute respiratory distress syndrome (ARDS) (ICD-10 code J80), acute respiratory failure (ICD-10 code J96.00), or pneumonia of unspecified organism (ICD-10 code J18.9) were included. In the remainder of this manuscript, this diagnostic group is identified as having COVID-19 respiratory complications. Individuals under 18 years of age and those without a diagnosis of COVID-19 pneumonia or COVID-19 plus pneumonia of an unspecified organism or respiratory failure were excluded.

The primary exposure was the hospital census region, as defined by the US Census Bureau. There are four census regions: Northeast, Midwest, South, and West (Figure 1) [10].

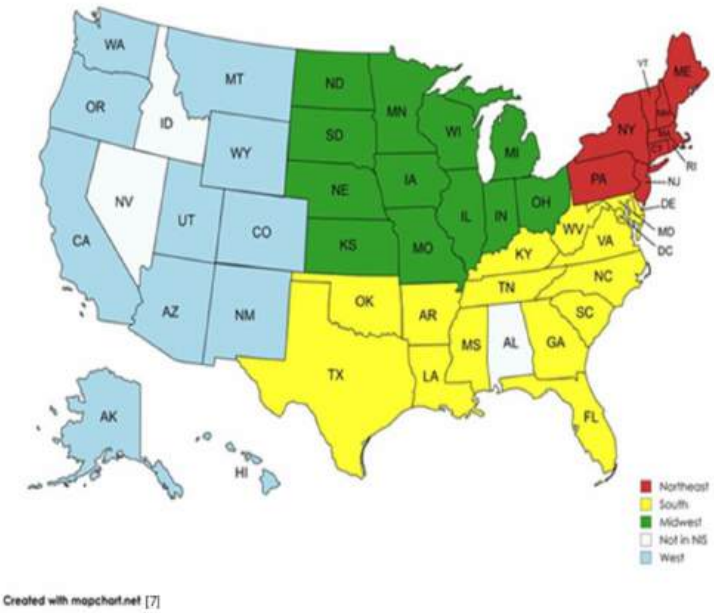


Figure 1. National Inpatient Sample by Census Region

The outcome of interest was in-hospital mortality. Other predictors included in the regression model were race, sex, expected primary payer of healthcare services, median household income for patient's ZIP code, Charlson Comorbidity Index (CCI), All Patient Refined Diagnostic Related Group (APRDRG) severity-of-illness subclass, age category, hospital ownership/control, and hospital location/teaching status [11,12].

Race data were extracted from patient records and administrative databases documenting patients' self-reported races. The sex of the patients, as provided by the data source, was captured in the NIS, with male and female categories. Any values outside of these categories were recorded as missing values. Additionally, instances in which the indicated sex did not align with other medical records or coding systems were considered inconsistent. Processing practices can vary by state, reflecting differences in confidentiality concerns and data handling guidelines [13]. Expected primary payer information is collected from patient records to identify the primary payer responsible for the healthcare services. The median household income for a patient's ZIP code was derived from the Claritas database, which provides income data relevant to patients' geographic areas [14]. The CCI was calculated based on comorbid conditions documented in medical records, whereas the APRDRG severity-of-illness subclass was assigned using the severity of illness and risk of mortality indicators from the APRDRG coding [11,15]. Hospital location and teaching status were assessed based on geographic location and teaching status, respectively.

Efforts to address the potential sources of bias in this study were systematically implemented. Rigorous diagnostic inclusion criteria were applied to select the patients. Standardized data collection was performed by the Healthcare Cost and Utilization Project, which employs reliable and validated methodologies designed to minimize bias [16]. Data analysis techniques, such as regression modeling, were employed to adjust for potential confounding variables, including demographic factors, comorbidities, and hospital characteristics.

In the analyses, quantitative variables were handled using appropriate statistical methods, considering their diverse nature. Age was categorized into three groups: 18–44 years, 45–64 years, and 65 years or older, reflecting distinct age brackets commonly used in healthcare research. Median household income was also treated as a categorical variable and was divided into quartiles to capture variations in socioeconomic status of participants across different patient populations. The CCI was treated as a continuous variable, enabling a detailed assessment of patient comorbidities. Additionally, the APRDRG severity-of-illness subclass was categorized into four groups: no or minor loss of function, moderate, major, and extreme, reflecting the severity of the patients' conditions. These groupings were chosen to facilitate meaningful comparisons and interpretation of the study results, while capturing the complexity of patient and hospital characteristics.

The statistical methods employed in this study primarily involved multivariate logistic regression to examine the relationships among the dependent variable (in-hospital mortality) and various independent variables. To control for confounding factors, demographic factors, including age, race, and sex, as well as comorbidities and hospital characteristics, were included as covariates in the regression model.

Missing data were handled by assuming that the missingness type was random. With missing data among variables at less than 3%, available case analysis was utilized for modeling purposes. As part of the sensitivity analysis, we employed multiple imputations via chained equations and utilized multinomial logistic regression to manage the categorical aspect of the race variable. In addition, a sensitivity analysis using multinomial logistic regression was employed to explore subgroup variations and assess the relationship between predictor variables and census regions. This statistical model facilitated a comprehensive analysis of demographic factors, such as race, sex, socioeconomic status, and comorbidities in relation to healthcare outcomes across diverse geographical areas. Statistical analyses were performed using STATA software version 18 MP software (College Station, TX, USA). A two-sided p-value of < 0.05 was taken to signify statistical significance.

In this study, analytical methods were tailored to accommodate the complex survey design of the NIS, ensuring accurate estimation at the national level by addressing its stratification, clustering, and weighting. To achieve this, the study utilized "survey data analysis modules" within statistical software packages, ensuring that the intricate survey design features were taken into consideration during statistical analyses.

The Institutional Review Board of Ponce Health Sciences University determined that the study (protocol ID # 2403189550) qualified for exemption of ethical review because it involved minimal risk, and classified it under the exempt category of research, thereby eliminating the need for obtaining informed patient consent.

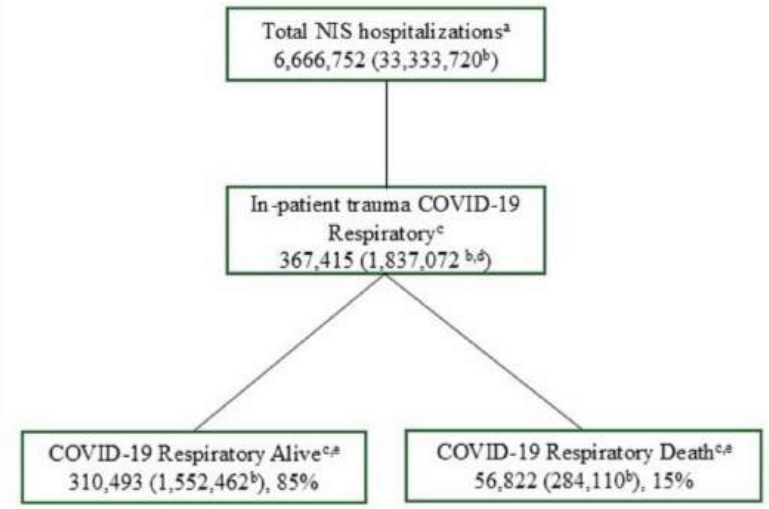
WordTune Editor and ChatGPT (version GPT-4) were used to enhance the manuscript's grammar and clarity of expression [17,18]. The manuscript was prepared following the methodology outlined by EQUATOR Network's Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) [19]. The STROBE checklist is provided in Supplementary Material.

3. Results

From January 1, 2021 to December 31, 2021, 6,666,752 hospitalizations were included in the NIS data, leading to a national estimate of 33,333,720 patient admissions after applying the relevant weights (Figure 2). However, this assumption may not be entirely accurate, as some individuals may have been hospitalized multiple times within a given period. Therefore, the actual number of unique individuals may have been lower than the estimated number. The data included 367,415 individuals aged 18 years or older with COVID-19 respiratory complications, which corresponds to a national estimate of 1,837,072 (Figure 2). Some individuals were not included into the study cohort due to the age restriction, whereas others were not tested for COVID-19 because of logistical constraints or limited access to testing resources. Additionally, instances of

196 mortality before test result availability and logistical barriers to testing contributed
197 to non-inclusion.

198 **Figure 2.** National Inpatient Sample patient flow-diagram for this study



a. Hospitalizations are treated as a distinct patient encounter (see methods section for details)
b. ( ) = National estimates
c. Covid-19 respiratory includes: Covid pneumonia, respiratory failure or pneumonia of unspecified organism with confirmed Covid 19
d. 18 years or older
e. Available case analysis

199

200 The study encompassed four distinct regions: Northeast, Midwest, South,
201 and West. These regions exhibited variations in patients' admissions, COVID-19
202 respiratory complications, and mortality rates (Table 1). The South region had the
203 highest percentage of admissions, accounting for 40% of the total, and the highest
204 number of cases with COVID-19 respiratory complications among all regions,
205 comprising 6.1% of these cases. In contrast, the Northeast region had the lowest
206 percentage of admissions, representing 18% of the total, and the lowest number
207 of cases, accounting for 4.5% of cases. Mortality rates among COVID-positive
208 cases ranged from 14% in the Midwest to 18% in the West. However, the age
209 distribution showed a consistent trend across regions, with the majority of cases
210 occurring in individuals aged 65 years and older. Sex distribution, payer
211 demographics, racial demographics, median household income, CCI, hospital
212 location/teaching status, APRDRG severity-of-illness subclass, and hospital
213 control (ownership) also showed notable variations across the four regions.

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219 **Table 1.** National Inpatient Sample (NIS) Demographics Using Sample Weights to
220 Achieve National Estimates

	Northeast		Midwest		South		West	
US Population (NIS)	57,259,257		68,836,505		122,296,183		73,539,047	
National admissions estimate	6,012,228 (18%)		7,267,247 (22%)		13,435,463 (40%)		6,618,782 (20%)	
COVID Respiratory Positive	271,254		354,475		818,603		342,974	
Died n, %	39,455	15%	48,090	14%	127,920	16%	60,115	18%
Age category n, %								
18–44 years	36,205	13%	58,195	15%	151,530	18%	65,090	18%
45–64 years	97,345	35%	141,620	38%	320,124	38%	133,205	38%
≥ 65 years	141,300	51%	176,070	47%	361,169	43%	155,219	44%
Sex								
Male	148,915	54%	199,320	53%	436,469	52%	199,324	56%
Female	125,920	46%	176,300	47%	396,294	48%	154,174	44%
Payer								
Medicare	138,935	51%	182,000	49%	359,814	43%	150,759	43%
Medicaid	42,360	15%	45,710	12%	74,740	9%	79,000	22%
Private insurance	80,450	29%	123,695	33%	293,659	35%	101,660	29%
Self-pay	4,835	2%	11,615	3%	46,880	6%	7,560	2%
No charge	600	0.2%	215	0.1%	3,380	0.4%	120	0.03%
Other	6,800	2%	11,655	3%	52,910	6%	13,865	4%
Race								
White	174,069	64%	274,185	77%	484,349	59%	173,404	51%
Black	38,280	14%	49,335	14%	170,295	21%	19,905	6%
Hispanic	32,620	12%	19,165	5%	124,375	15%	107,740	31%
Asian or Pacific Islander	9,985	4%	5,360	2%	11,410	1%	23,490	7%
Native American	475	0.2%	1,885	1%	4,085	1%	6,900	2%
Other	15,895	6%	4,560	1%	24,260	3%	11,750	3%
Median household income for patient's ZIP code								
0–25th percentile	58,085	21%	113,385	30%	336,114	41%	75,035	22%
26th–50th percentile	64,130	24%	118,015	32%	227,410	28%	86,365	25%
51st–75th percentile	73,565	27%	95,390	26%	158,565	19%	102,750	30%
76th–100th percentile	75,425	28%	46,675	12%	97,415	12%	79,880	23%
Charlson Comorbidity Index (95%	1.92	(1.878–1.956)	1.96	(1.930–1.987)	1.75	(1.722–1.770)	1.79	(1.761–1.819)

confidence interval)								
Location/teaching status of hospital								
Rural	20,000	7%	65,410	17%	107,359	13%	25,144	7%
Urban nonteaching	27,795	10%	63,844	17%	210,525	25%	89,315	25%
Urban teaching	227,055	83%	246,631	66%	514,939	62%	239,055	68%
APRDRG severity-of-illness subclass								
None to Minor	30	<0.001%	25	<0.001%	135	<0.001	25	<0.001%
Moderate	1,625	1%	1,065	<0.001%	4,270	1%	1,210	<0.001%
Major	73,475	27%	87,920	23%	182,204	22%	57,905	16%
Extreme	199,719	73%	286,875	76%	646,214	78%	294,374	83%
Hospital Control								
Government, nonfederal	13,625	5%	28,409	8%	122,739	15%	39,839	11%
Private, non-profit	252,594	92%	329,066	88%	524,049	63%	256,505	73%
Private, investor-owned	8,630	3%	18,410	5%	186,035	22%	57,170	16%

APRDRG, All Patient Refined Diagnostic Related Group

Regarding payer demographics, Medicaid emerged as a significant payer across all regions, although with varying proportions. In the Northeast, Medicaid covered 15% of cases, whereas private insurance covered 29%. The Midwest had similar proportions, with Medicaid covering 12% of cases and private insurance covering 33%. In the South, Medicaid covered 9% of cases, while private insurance covered 35%. The West saw a slight shift, with Medicaid covering 22% and private insurance covering 29% of cases.

Analysis of racial demographics revealed distinct patterns among the regions. While the Northeast and Midwest regions had predominantly White populations, the South exhibited greater diversity, with a notable proportion of Black and Hispanic patients. In contrast, the West displayed a more balanced distribution of White and Hispanic patients. The South had the highest concentration of patients in the lowest income bracket (41%) and tied with the Midwest in having the lowest proportion of cases in the highest income bracket (76th–100th percentile) at 12%.

The dataset exhibited varying levels of missingness across different variables. Among the variables examined, the highest proportion of missing data was observed for the race variable, with 2.68% of entries missing. This was followed by median household income (1.57% missing) and payer information (0.21% missing). Other variables, such as death and sex, exhibited minimal missing observations, with only 0.03% and 0.02% missing values, respectively.

Multivariate logistic regression analysis revealed significant differences in COVID-19 respiratory complication mortality rates across regions, even after adjusting for potential confounding variables (Table 2). In the Northeast, individuals with COVID-19 had approximately 10% higher odds of experiencing COVID-19 respiratory complication-related deaths than those in the Midwest (reference group; odds ratio [OR] 1.10, 95% confidence interval [CI]: 1.038–1.165, $p = 0.001$). In the South, individuals had approximately 21.0% higher odds of COVID-19 respiratory complication-related deaths than the reference group (OR 1.21; 95%CI: 1.159–1.272; $p < 0.001$). Additionally, individuals in the West had approximately 28% higher odds of COVID-19 respiratory complication-related deaths than those in the reference group (OR 1.28; 95%CI: 1.218–1.354; $p < 0.001$).

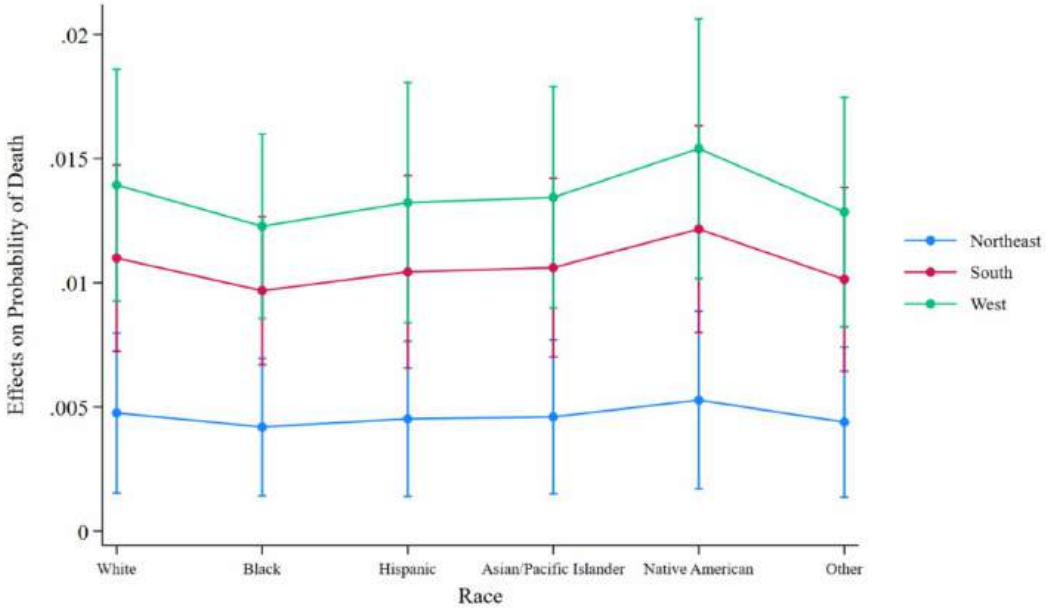
256 **Table 2.** Multivariate Logistic Regression with Death as Outcome Variable

	Odds ratio	Std. Error	p=value	95% Conf. Interval	
Hospital region					
Midwest (reference)					
Northeast	1.10	0.032	0.001	1.038	1.165
South	1.21	0.029	<0.001	1.159	1.272
West	1.28	0.035	<0.001	1.218	1.354
Race					
White (reference)					
Black	0.91	0.017	<0.001	0.877	0.941
Hispanic	1.18	0.023	<0.001	1.138	1.227
Asian or Pacific Islander	1.10	0.038	0.004	1.033	1.181
Native American	1.34	0.080	<0.001	1.188	1.503
Other	1.08	0.036	0.018	1.014	1.154
Sex					
Male (reference)					
Female	0.81	0.008	<0.001	0.791	0.823
Payer					
Medicare (reference)					
Medicaid	1.12	0.025	<0.001	1.074	1.170
Private Insurance	0.92	0.017	<0.001	0.887	0.955
Self-Pay	1.07	0.040	0.085	0.991	1.148
No Charge	1.02	0.124	0.845	0.807	1.299
Other	1.36	0.049	<0.001	1.266	1.459
Median household income for patient's ZIP code					
0–25th percentile (reference)					
26th–50th percentile	0.88	0.014	<0.001	0.856	0.910
51st–75th percentile	0.82	0.014	<0.001	0.794	0.851
76th–100th percentile	0.73	0.016	<0.001	0.696	0.758
Charlson Comorbidity Index	1.14	0.003	<0.001	1.133	1.144
Age category					
18–44 years (reference)					
45–64 years	2.11	0.044	<0.001	2.026	2.199
≥ 65 years	3.81	0.095	<0.001	3.628	4.002
Hospital Control					
Government, nonfederal (reference)					
Private, non-profit	0.90	0.025	<0.001	0.852	0.950
Private, investor-own	0.91	0.032	0.006	0.848	0.972
Location/teaching status of hospital					
Rural (reference)					
Urban nonteaching	1.09	0.034	0.006	1.024	1.156

Urban teaching	1.21	0.033	<0.001	1.151	1.280
Severity-of-illness subclass					
None to minor (reference)					
Moderate	0.11	0.091	0.007	0.023	0.549
Major	0.57	0.433	0.462	0.131	2.523
Extreme	2.61	1.970	0.204	0.595	11.460

When examining the average marginal effects (AVEs) of COVID-19 respiratory complication mortality rates across different racial groups and regions, consistent patterns emerged (Figure 3). For all racial groups, an increase in the probability of COVID-19 respiratory complication-related deaths was observed across all regions as compared with the reference region (Midwest). Although the magnitude of this increase varied slightly across racial groups, the overall trend remained consistent across regions. Notably, the South and West consistently exhibited higher AVEs for all racial groups than did the Northeast, indicating heightened risks of COVID-19 respiratory complication-related mortality in these regions. Despite variations in the absolute values, the disparities in COVID-19 respiratory complication-related mortality rates appeared to be consistent across regions for all racial groups analyzed.

Figure 3. Average Marginal Effects of Regions, with 95% Confidence Intervals, Against the Midwest Region as Reference



A sensitivity analysis was conducted for multiple imputations of the race variable but revealed no discernible disparities in outcomes when compared to the regression analysis utilizing all available data. Additionally, as part of the sensitivity analysis, multinomial logistic regression was used to compare the regions, with the Midwest serving as the reference group. Significant differences emerged across the regions in terms of race, payer status, median income, hospital control, and hospital teaching status. Detailed findings from this sensitivity analysis are provided in the supplemental documents accompanying this study (Tables S2 and S3).

4. Discussion

In this study, regional disparities in COVID-19 outcomes, particularly in-hospital mortality among COVID-19 patients with pneumonia or respiratory failure, were examined using the 2021 NIS database in the USA. Significant

regional disparities in COVID-19 outcomes across the USA were revealed by the findings, with variations in hospitalization rates, COVID-19 respiratory complication rates, and related mortality rates observed in the Northeast, Midwest, South, and West regions. Notably, the highest proportion of COVID-19 respiratory complications was observed in the South, while the lowest proportion was observed in the Northeast. Mortality rates ranged from 14% in the Midwest to 18% in the West. Particularly noteworthy results emerged from the West, where up to 28% higher odds of COVID-19 respiratory complication-related death were revealed by regression analysis compared to the Midwest, indicating a pronounced disparity in mortality rates. The necessity of employing advanced analytical techniques to adjust for confounding factors is underscored by these findings.

Previous studies examining regional differences in outcomes across various diseases, including COVID-19, have consistently reported similar disparities [20-24]. For instance, research has shown that regions with lower socioeconomic status and limited access to healthcare facilities often experience higher mortality rates and worse health outcomes [1]. These findings align with the observed regional variations in COVID-19 outcomes, suggesting that the underlying systemic factors play a crucial role in shaping health disparities. Furthermore, studies focusing specifically on COVID-19 have highlighted the influence of factors such as population density, healthcare infrastructure, and public health policies in driving regional differences in the outcomes of this disease [25-28].

The analysis aligns with a robust body of literature that highlights disparities across various health conditions and regional constructs within the United States. These disparities manifest in diverse ways, influenced by factors such as healthcare utilization patterns, racial disparities in healthcare access, differential healthcare expenditures, and specific health conditions [29-32].

For instance, studies consistently show significant regional disparities in healthcare outcomes for conditions such as cardiovascular disease, cancer, and diabetes, where access to specialized care and treatment options varies across different geographic regions[33-35]. Additionally, disparities in healthcare utilization and outcomes have been documented among racial and ethnic minority populations, often due to systemic barriers to accessing quality healthcare services [36,37] .

By contextualizing these findings within the broader literature, this study contributes to a deeper understanding of how regional disparities manifest across various health conditions and emphasizes the ongoing need for targeted interventions to promote equitable healthcare access and improve health outcomes across diverse regional landscapes.

To mitigate these disparities, interventions such as enhancing healthcare infrastructure in underserved areas, implementing targeted public health campaigns for minority populations, expanding Medicaid coverage, and fostering community partnerships are crucial. These interventions aim to improve access to quality care and promote equity in healthcare outcomes.

Despite its comprehensive nature, this study had several limitations that must be acknowledged. First, the reliance on administrative databases entails inherent biases and limitations in retrospective analyses, potentially causing inaccuracies in data interpretation [38]. Furthermore, the scope of the study was limited to individuals who had undergone COVID-19 testing, potentially excluding those who did not seek testing or were unable to access healthcare services, thereby limiting the generalizability of the findings. Additionally, the retrospective design of the study precluded the establishment of causal relationships between the variables, necessitating cautious interpretation of the results. Moreover, although efforts have been made to adjust for confounding factors using advanced statistical techniques, the possibility of residual confounding remains, which may influence the observed associations. Finally, the study's reliance on data from a single year [3] limits its ability to assess temporal trends and longitudinal changes in COVID-19 outcomes over time. Despite these limitations, this study provided valuable insights into the regional disparities in COVID-19 outcomes, highlighting the need for further research to address these

disparities and inform targeted interventions aimed at promoting health equity across diverse populations.

The NIS provides a robust foundation for examining regional disparities in COVID-19 outcomes, offering insights into diverse patient populations across the USA. However, since the study utilized national estimates from the NIS, its findings can be applied across all non-federal, short-term, general, and specialty community hospitals in the 47 US states and Washington, DC [39]. Although efforts were made to ensure the representativeness of the sample through meticulous weighting procedures, caution should be exercised when extrapolating the results to populations that are not captured within the NIS framework, such as individuals treated in long-term care facilities or outpatient settings. Despite these considerations, the NIS remains a valuable resource for understanding healthcare trends and disparities at the national level, providing a foundation for further investigations of regional variations in COVID-19 outcomes.

5. Conclusions

In conclusion, by employing a rigorous regression analysis, this study shed light on the significant regional disparities in COVID-19 outcomes across the USA, emphasizing the pronounced differences in mortality rates observed in the Western region. The findings of this study underscored the importance of understanding and addressing regional nuances in healthcare outcomes and highlight the significance of targeted interventions to mitigate disparities and promote health equity in diverse populations. Continued research on regional differences is essential to inform evidence-based policies and interventions to improve healthcare outcomes and resilience in the face of future health crises.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: Supplemental 1. STROBE Statement: Checklist of items that should be included in reports of observational studies; Table S2. Multiple Imputation for Race; Table S3. Multinomial Logistic Regression Analysis by Region.

Author Contributions: The author framed the study objectives, established the research methodology, processed and analyzed the data, and wrote the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Ethical review and approval were waived for this study by the Institutional Review Board of Ponce Health Sciences University because it involved minimal risk and was classified under the exempt category of research.

Informed Consent Statement: The need for informed consent was waived by the Institutional Review Board of Ponce Health Sciences University.

Data Availability Statement: The data supporting the findings of this study can be accessed from Healthcare Cost and Utilization Project at https://hcup-us.ahrq.gov/.

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Table S1. STROBE Statement: Checklist of items that should be included in reports of observational studies.

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1,2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2,3
Objectives	3	State specific objectives, including any prespecified hypotheses	2,3
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5,6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7,8
		(*b*) Describe any methods used to examine subgroups and interactions	7,8
		(*c*) Explain how missing data were addressed	7,8
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	7

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	8
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—e.g., numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analyzed	9
		(b) Give reasons for non-participation at each stage	9
		(c) Consider use of a flow diagram	9
Descriptive data	14*	(a) Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential confounders	11,12
		(b) Indicate number of participants with missing data for each variable of interest	13
		(c) *Cohort study*—Summarize follow-up time (e.g., average and total amount)	N/A
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	N/A
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	N/A
		Cross-sectional study—Report numbers of outcome events or summary measures	11,12
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included	13,14
		(*b*) Report category boundaries when continuous variables were categorized	14,15
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	16
Discussion			
Key results	18	Summarize key results with reference to study objectives	16,17
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17,18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	16-18
Generalizability	21	Discuss the generalizability (external validity) of the study results	18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	N/A

Table S2. Multiple Imputation for Race

	Odds ratio	Std. Error	p-value	95% Conf. Interval	
Hospital region					
Midwest reference					
Northeast	1.09	0.032	0.002	1.031	1.156
South	1.21	0.028	<0.001	1.153	1.264
West	1.29	0.034	<0.001	1.223	1.357
Race					
White reference					
Black	0.91	0.016	<0.001	0.880	0.944
Hispanic	1.18	0.023	<0.001	1.132	1.221
Asian or Pacific Islander	1.10	0.037	0.004	1.031	1.176
Native American	1.34	0.080	<0.001	1.193	1.508
Other	1.08	0.036	0.018	1.014	1.155
Female	0.81	0.008	<0.001	0.792	0.824
Payer					
Medicare reference					
Medicaid	1.12	0.024	<0.001	1.075	1.170
Private Insurance	0.92	0.017	<0.001	0.889	0.956
Self-Pay	1.07	0.039	0.066	0.996	1.149
No Charge	1.01	0.122	0.922	0.799	1.282
Other	1.36	0.049	<0.001	1.265	1.456
Median household income for patient's ZIP code					
0–25th percentile reference					
26th–50th percentile	0.88	0.014	<0.001	0.858	0.911
51st–75th percentile	0.82	0.014	<0.001	0.793	0.849
76th–100th percentile	0.72	0.015	<0.001	0.693	0.754
Charlson Comorbidity Index	1.14	0.003	<0.001	1.133	1.144
Age category					
18– 44 years (reference)					
45–64 years	2.09	0.043	<0.001	2.011	2.179
65 years and older	3.78	0.093	<0.001	3.606	3.972
Hospital Control					
Government, nonfederal (reference)					
Private, non-profit	0.90	0.025	<0.001	0.855	0.952
Private, investor-own	0.91	0.031	0.007	0.852	0.974
Location/teaching status of hospital					
Rural reference					
Urban nonteaching	1.09	0.033	0.004	1.028	1.159
Urban teaching	1.22	0.033	<0.001	1.156	1.284
Severity-of-illness subclass					
None to minor (reference)					
Moderate	0.11	0.089	0.007	0.022	0.542

Major	0.58	0.435	0.466	0.131	2.534
Extreme	2.63	1.981	0.201	0.598	11.522

Table S3. Multinomial Logistic Regression Analysis by Region

Region	Variable	Relative risk ratio	Standard error	p-value	95% conf. interval	
Northeast						
	Race	White (reference)				
	Black	1.27	0.116	0.008	1.066	1.522
	Hispanic	2.87	0.291	<0.001	2.355	3.502
	Asian or Pacific Islander	2.38	0.374	<0.001	1.752	3.243
	Native American	0.52	0.099	0.001	0.361	0.759
	Other	5.03	0.790	<0.001	3.698	6.846
	Female	0.94	0.012	<0.001	0.922	0.968
	Payer	Medicare (reference)				
	Medicaid	1.43	0.057	<0.001	1.322	1.547
	Private Insurance	0.94	0.039	0.132	0.866	1.019
	Self-Pay	0.53	0.059	<0.001	0.429	0.664
	No Charge	3.61	1.413	0.001	1.674	7.774
	Other	0.84	0.075	0.046	0.702	0.997
	Median household income for patient's ZIP code	0–25th percentile (reference)				
	26th– 50th percentile	1.10	0.083	0.187	0.953	1.280
	51st–75th percentile	1.43	0.136	<0.001	1.184	1.721
	76th–100th percentile	2.93	0.384	<0.001	2.266	3.787
	Charlson Comorbidity Index	0.97	0.005	<0.001	0.958	0.978
	Age category	18–44 years (reference)				
	45–64 years	1.30	0.030	<0.001	1.247	1.364
	65 years and older	1.74	0.070	<0.001	1.606	1.880
	Hospital Control	Government, nonfederal (reference)				
	Private, non-profit	1.51	0.208	0.003	1.155	1.981
	Private, investor-own	1.03	0.222	0.909	0.670	1.567
	Location/teaching status of hospital	Rural (reference)				
	Urban nonteaching	0.94	0.114	0.596	0.738	1.191
	Urban teaching	2.00	0.210	<0.001	1.627	2.456
	Severity-of-illness subclass	None to Minor (reference)				
	Moderate	1.13	0.800	0.866	0.280	4.532
	Major	0.69	0.480	0.593	0.176	2.701
	Extreme	0.55	0.384	0.392	0.141	2.158
Midwest	(base outcome)					
South						
	Race	White (reference)				
	Black	1.95	0.147	<0.001	1.685	2.261
	Hispanic	3.08	0.277	<0.001	2.585	3.675

	Asian or Pacific Islander	1.24	0.111	0.014	1.046	1.482
	Native American	1.35	0.235	0.084	0.961	1.900
	Other	2.68	0.337	<0.001	2.091	3.424
	Female	1.04	0.011	0.001	1.014	1.058
	Payer	Medicare (reference)				
	Medicaid	0.66	0.028	<0.001	0.606	0.714
	Private Insurance	1.24	0.049	<0.001	1.152	1.345
	Self-Pay	1.73	0.145	<0.001	1.470	2.043
	No Charge	6.66	3.563	<0.001	2.331	19.010
	Other	2.07	0.144	<0.001	1.803	2.370
	Median household income for patient's ZIP code	0–25th percentile (reference)				
	26th–50th percentile	0.66	0.037	<0.001	0.592	0.737
	51st–75th percentile	0.57	0.042	<0.001	0.491	0.657
	76th–100th percentile	0.75	0.085	0.011	0.600	0.937
	Charlson Comorbidity Index	0.96	0.005	<0.001	0.952	0.970
	Age category	18–44 years (reference)				
	45–64 years	0.98	0.017	0.162	0.942	1.010
	65 years and older	1.11	0.038	0.002	1.040	1.188
	Hospital Control	Government, nonfederal (reference)				
	Private, non-profit	0.38	0.034	<0.001	0.321	0.455
	Private, investor-own	2.51	0.364	<0.001	1.890	3.335
	Location/teaching status of hospital	Rural (reference)				
	Urban nonteaching	2.03	0.178	<0.001	1.707	2.408
	Urban teaching	1.28	0.100	0.001	1.103	1.496
	Severity-of-illness subclass	None to Minor (reference)				
	Moderate	0.62	0.308	0.332	0.231	1.640
	Major	0.40	0.198	0.064	0.155	1.054
	Extreme	0.45	0.222	0.105	0.173	1.182
West						
	Race	White (reference)				
	Black	0.67	0.061	<0.001	0.562	0.803
	Hispanic	8.25	0.730	<0.001	6.934	9.810
	Asian or Pacific Islander	5.80	0.535	<0.001	4.837	6.945
	Native American	7.05	1.098	<0.001	5.195	9.568
	Other	3.39	0.480	<0.001	2.570	4.476
	Female	0.89	0.012	<0.001	0.872	0.918
	Payer	Medicare (reference)				
	Medicaid	1.83	0.078	<0.001	1.685	1.990
	Private Insurance	0.93	0.036	0.059	0.862	1.003
	Self-Pay	0.50	0.053	<0.001	0.408	0.616
	No Charge	0.55	0.273	0.228	0.209	1.453
	Other	1.19	0.096	0.032	1.015	1.395

	Median household income for patient's ZIP Code	0–25th percentile (reference)				
	26th–50th percentile	1.13	0.087	0.098	0.977	1.318
	51st–75th percentile	1.61	0.154	<0.001	1.333	1.941
	76th–100th percentile	2.75	0.346	<0.001	2.150	3.519
	Charlson Comorbidity Index	0.97	0.005	<0.001	0.964	0.984
	Age category	18–44 years (reference)				
	45–64 years	1.10	0.025	<0.001	1.055	1.153
	65 years and older	1.34	0.053	<0.001	1.243	1.451
	Hospital control	Government nonfederal reference				
	Private, non-profit	0.53	0.060	<0.001	0.427	0.666
	Private, investor-own	2.11	0.361	<0.001	1.507	2.950
	Location/teaching status of hospital	Rural (reference)				
	Urban nonteaching	2.25	0.280	<0.001	1.765	2.872
	Urban teaching	1.58	0.166	<0.001	1.283	1.937
	Severity-of-illness subclass	None to Minor (reference)				
	Moderate	1.49	1.211	0.621	0.305	7.325
	Major	1.15	0.920	0.858	0.242	5.510
	Extreme	1.74	1.386	0.488	0.364	8.298

Original Article

Robotically Assisted Minimally Invasive Transforaminal Lumbar Interbody Fusion (MIS-TLIF) Outcomes in the Aging Hispanic Population: A Retrospective Cohort Study

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Abstract: Minimally invasive techniques for lumbar spinal fusions have evolved significantly to treat lumbar spinal stenosis, degenerative spondylolisthesis, and many other complex conditions. This study evaluates the clinical outcomes of patients aged 65 and older who underwent minimally invasive transforaminal lumbar interbody fusion (MIS-TLIF) with robotic assistance. In this single-surgeon single-institution retrospective cohort study, 72 patients aged 65 and older who underwent MIS-TLIF from 2018 to 2021 were analyzed. Patients had diagnoses of lumbar spinal stenosis, with or without degenerative spondylolisthesis. Clinical outcomes were assessed using the Oswestry Disability Index (ODI) and Visual Analogue Scale (VAS) for back and lower extremities at baseline, and 6, 9, or 12 months postoperatively. The data was analyzed, and outcomes were compared using paired t-tests. Significant improvements in disability were observed postoperatively, with mean ODI scores decreasing from 46.4% to 9.3% (95% CI: -41.2, -33.1). In terms of pain intensity, mean VAS scores for back pain decreased from 8.0 to 3.5 (95% CI: -5.1, -3.9) and leg pain scores also decreased from 8.2 to 2.9 (95% CI: -5.9, -4.6). These changes indicate substantial clinical improvements ($p < 0.001$). This study substantiates the efficacy of MIS-TLIF in significantly improving pain relief and functional mobility among seniors with lumbar conditions. The substantial reductions in ODI and VAS scores highlight its clinical benefits potential to set a new standard of care. By offering a robotically assisted, minimally invasive alternative, this approach aligns with contemporary healthcare objectives of enhancing patient recovery while minimizing procedural risks and costs.

Keywords: MIS-TLIF; Robotic Spine Surgery; Lumbar Stenosis; Degenerative Spondylolisthesis; Elderly Patient Care; Spinal Fusion; Postoperative Outcomes; Quality of Life; Orthopedics

1. Introduction

Lumbar spinal fusion techniques have evolved throughout the past thirty-plus years to better stabilize and decompress unstable and stenotic vertebral segments. What started with open laminectomies and posterolateral fusions with freehand pedicle screw placement gradually progressed to minimally invasive approaches and interbody fusions with pre-planned, robotically navigated and assisted pedicle screw trajectories. From the first successful posterior lumbar interbody fusion (PLIF) in 1940, which used a spinous process autograft, to Blume in 1981, who described a unilateral approach to the already established PLIF procedure. Subsequently, Harms popularized the open TLIF (oTLIF)

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PLIF procedure. Subsequently, Harms popularized the open TLIF (oTLIF) technique in the late 1990s, a posteriorly based open lumbar fusion with or without interbody support. The oTLIF has been the workhorse for spine surgeons throughout the past three to four decades [1-3]. By the 2000s, the minimally invasive transforaminal lumbar interbody fusion (MIS TLIF) came to fruition. It drew significantly from the paraspinal sacrospinalis splitting approach between the multifidus and longissimus muscles, first described by Wiltse in 1988. The MIS TLIF revolutionized and reinvented the practices of many spine surgeons around the country. The term "minimally invasive surgery" was popularized in part due to the significant improvement in the acute postoperative period regarding decreased tissue dissection and morbidity. It also reduced blood loss, infection rate, length of stay, and overall complication rate. The benefits of minimally invasive approaches go further as they also result in cost savings for the healthcare system's secondary to decreased length of stay (LOS), need for transfusion, lower inpatient resource utilization such as inpatient rehabilitation, and narcotic medication overuse [4].

The elderly population in the United States increases every year. In 2010, there were 11.2 million Americans aged 80 years and over [5]. This is a 22% increment in this subgroup compared to the year 2000. According to the American Community Survey (ACS), in 2016, the estimated number of people in the United States aged 65 and over was 49.2 million. Of these, 58% were aged 65 to 74; 29% were between the ages of 75 to 84, and 13% were 85 and older [6]. This population subgroup commonly presents with lumbar spinal stenosis (with or without neurogenic claudication), mechanical lower back pain (with or without radicular symptoms), multilevel degenerative disc disease, and degenerative spondylolisthesis. These findings are found both clinically and with lumbar MRI use. To better illustrate this, a 2009 study evaluating cases that underwent computed tomography (CT scan) showed that 47% of patients who were 60 years of age and older had lumbar spinal stenosis [3,7].

The number of spinal procedures performed on the elderly, particularly fusion procedures, increases every year [8]. The four-year outcome data in the Spine Patients Outcomes Research Trial (SPORT) shows that the operative treatment of lumbar spinal stenosis and degenerative spondylolisthesis provides better long-term benefits than non-operative management. Further, it is common knowledge that healthcare funds are scarce, and payers are demanding better quality of care for compensation provided. Therefore, there is a need for a consensus on the most effective approach and surgical technique for lumbar spinal stenosis in this age group. Most spine surgeons in Puerto Rico opt for open laminectomy and posterolateral fusion with or without interbody fusion as their technique of choice in this aging population. Alternatively, they also rely on indirect decompressions via an oblique lumbar interbody fusion (OLIF) or a lateral lumbar interbody fusion (LLIF), particularly when instrumented fusions are needed. The present study aims to assess clinical outcomes for patients aged 65 years and over who underwent a minimally invasive robotically assisted transforaminal lumbar interbody fusion (MIS TLIF) at one or two lumbar levels.

2. Methodology

After receiving approval from the Institutional Review Board at Ponce Health Sciences University, we conducted a retrospective analysis at Centro Médico Menonita in Cayey, Puerto Rico. We included patients aged 65 and over requiring lumbar fusion at one or two lumbar levels between August 2018 and December 2021. A total of 72 patients matched the inclusion criteria. Patients aged 64 and under, trauma patients, grade 3 spondylolisthesis or greater, 100 % axial back pain patients, patients with prior instrumented fusions, and patients undergoing lumbar decompression without implants (i.e., laminoforaminotomy) were excluded.

None of the patients in this study were active smokers, and nearly all had BMIs of 35 or less, with a few exceptions due to severity of pathology and symptoms. All patients presented with axial low back pain, unilateral or bilateral radicular leg symptoms, and neurogenic claudication. All patients underwent conservative non-operative management, including but not limited to physical

therapy, oral medications, and pain management blocks by a pain management specialist for at least six months prior to opting for a procedure. The data collected from the hospital medical records for this study included: age, sex, comorbidity variables (hypertension, obesity, active smoking status, diabetes mellitus, etc.), and information pertaining to the procedure (length of surgery, estimated blood loss, complications, location, and number of vertebral segments operated).

As part of an established preoperative planning protocol in this practice, all patients provided standing lumbar spine X-rays AP/LAT and flexion/extension views, CT lumbar with axial/sagittal/coronal reconstructions, and a DEXA scan showing bone density greater than -2.5 score in the lumbar spine to confirm candidacy for an instrumented fusion. Additionally, all patients needed a complete laboratory workup, including a CBC with differential, CMP, coagulation profile, HbA1c less than 7%, Vitamin D levels of 30-50 ng/mL, urine analysis, urine culture, serum albumin levels between 3.5 to 5.4 g/dL, transferrin levels between 200 to 360 mg/dL. The preoperative protocol also required an EMG-nerve conduction study, outpatient cardiology clearance, and hospital pre-admission evaluation and clearance.

For the evaluation of surgical success, the clinical outcomes were evaluated based on the improvement of back and leg pain individually, the degree of preoperative versus postoperative functional disability, and whether there were any perioperative complications. Patients' back and leg pain levels were evaluated using a ten-point Visual Analogue Scale (VAS). VAS scores range from 0 to 10, with lower scores indicating less severe pain symptoms. The Oswestry Disability Index (ODI) was used to quantify preoperative and postoperative disability. ODI scores range from 0 to 50, with lower scores indicating less disability. These ODI scores are then calculated into ODI percentages, the unit used in most studies and will be referenced in this study as well. Patients were interviewed in person or by phone to assess long-term VAS and ODI scores. Clinical outcomes were evaluated at baseline preoperatively and postoperatively at 6, 9, and 12-month marks.

All of the lumbar fusions are performed via two paraspinal Wiltse incisions to perform an MIS TLIF technique regardless of age or severity of pathology. All cases were instrumented with robotic assistance using Medtronics cannulated Solera/Voyager MIS corticocancellous pedicle screws via guided K-wires and FUSE titanium interbody cage instrumentation. Patient autograft was utilized from a medial facetectomy, BMP-2 (Infuse, Medtronic Sofamor Danek, Memphis, Tennessee; 1.4mL per level), demineralized bone graft, and 20-30cc of cancellous bone chips per level. The aforementioned were placed through a quadrant retractor system attached to a fixed table arm. Medtronics O-arm and Mazor X robotic technology (without stealth navigation edition) were used to plan screw trajectories and customize pedicle screw sizes for improved accuracy. All screws were probed with neuromonitoring, and all yielded satisfactory results. Lateral fluoroscopy was used to confirm correct screw placement and to ensure all inserted instruments, including disc pre-instrumentation, were in a safe area. All patients underwent post-instrumentation O-arm spin to corroborate preplanned robotic software screw projections to the real-time screw trajectories seen on CT image quality. For those patients with a personal history of cancer, we used Nuvasive Osteocel (5cc) instead of BMP-2. All patients were pre-medicated with tranexamic acid (20mg/kg loading dose and 4mg/kg infusion until wound closure; dose adjusted for cardiac and renal disease patients). Also, all patients were given 2g cefazolin IV every three hours and 1g vancomycin if allergic to penicillin. Prior to closure, all wounds were irrigated with diluted iodine (35mL in 1000mL saline preparation) and three additional liters of normal saline and cefazolin or bacitracin prior to interbody insertion. Vancomycin powder was placed subfascial and suprafascial inside all wounds prior to closure as long as the patient did not have a vancomycin allergy. If there was a dural tear during the procedure, vancomycin was only placed suprafascial. All wounds were closed in layers with Vicryl 1 and 2-0 undyed, and finally, subcuticular closure with Quil 2-0. Dermabond was then placed on all surgical wounds, followed by adequately sized dressings.

Statistical analysis was conducted using MedCalc software. Initially, descriptive statistics were applied to summarize demographic and clinical variables such as age, sex, comorbidities, and surgical details, providing an overview of the patient cohort. To assess changes in clinical outcomes, two-sided paired t-tests were used to compare preoperative and postoperative scores of the ODI and VAS scales. The ODI scores were analyzed in percentage terms to reflect the extent of disability reduction, while VAS scores were evaluated to measure changes in pain intensity. We accepted p-values below 0.05 as indicative of statistical significance. Results were reported with 95% confidence intervals to quantify the precision of the observed effects.

3. Results

Patient Demographics and Surgical Details: A total of 72 (35 male and 37 female) patients with a mean age of 71 years [65-85 range] fulfilled the inclusion criteria and were included in the study. Most patients had a preoperative diagnosis of lumbar spinal stenosis with radiculopathy. The most stenotic level was L4-L5 in 41 patients (56.9%), followed by L5-S1 in 24 patients (33.3%), L3- L4 in five patients (6.9%), and two patients (2.9%) were surgically intervened at the L2-L3 level. Sixty-eight patients (94.4%) underwent single-level MIS TLIF, and four (5.6%) underwent 2-level MIS TLIF. Diabetes mellitus, hypertension, chronic kidney disease, asthma, obesity, and thyroid disease were the comorbidities that showed the most prevalence (Table 1).

Table 1. Prevalence of Comorbidities Among Study Participants

Prevalence of Comorbidities	*n* (%)
Asthma	8 (11.1%)
Chronic Kidney Disease	2 (2.8%)
Diabetes Mellitus	36 (50%)
Hypertension	54 (75%)
Obesity	3 (4.2%)
Thyroid Disease	17 (23.6%)
Ex-Smoker (History of tobacco use)	26 (36.1%)

This table summarizes the frequency and percentage of common comorbidities found in the study cohort of 72 patients undergoing minimally invasive transforaminal lumbar interbody fusion. The data highlight the prevalence of each condition to contextualize the health profile of the patient population.

Surgical Outcomes and Complications: The mean surgical time for a single level, robotically assisted MIS TLIF was 292.4 minutes. The mean surgical time for a two-level, robotically assisted MIS TLIF was 466.4 minutes. The average estimated blood loss (EBL) was minimal at 78.8 ccs (SD=15.51). The average length of stay was 1.1 days [range 1-5 days], with only two patients discharged home after 24 hours. These two prolonged hospitalizations were due to (1) a blood transfusion which delayed discharge and (2) the need for an additional day in the hospital for pain control. Three events were categorized as surgical complications (3 of 72 cases; 4.2% complication rate). These included a blood transfusion to resolve a hemoglobin value of 8.0, a seroma formation with pedicle screw misplacement, and a small dural tear that was repaired primarily. The misplaced pedicle screw was inserted 4mm too medial out of 296 total screws placed robotically. This indicates a 0.34% hardware revision rate. None of the three complications resulted in any long-term consequences for the patients, and none of the patients in this study necessitated inpatient rehabilitation. All patients were able to return home from the hospital with the only directives being (1) to ambulate one mile per day and (2) progress as tolerated with stretching at six weeks. None of the patients in this study had an infection or rule-out thereof.

Oswestry Disability Index: The study demonstrated significant improvements in ODI scores from preoperative to postoperative assessments. Initially, the mean preoperative ODI score (Table 2) was 46.4% (SD = 17.3), indicating a moderate to severe disability level among participants. This score significantly decreased to 9.3% (SD = 7.6) postoperatively, reflecting a substantial reduction in patient-reported disability. The mean difference observed was -37.1 percentage points (95% CI: -41.2, -33.1, $p < 0.0001$).

Visual Analogue Scale: Similarly, significant improvements were noted in pain severity as measured by the VAS for both back and leg pain. The mean back pain score (Table 2) decreased from 8.0 (SD = 2.8) preoperatively to 3.5 (SD = 2.3) postoperatively, with a mean difference of -4.5 points (95% CI: -5.1, -3.9, $p < 0.0001$). Leg pain scores (Table 2) also showed considerable improvement, decreasing from 8.2 (SD = 2.5) to 3.5 (SD = 2.6), resulting in a mean difference of -5.2 points (95% CI: -5.9, -4.6, $p < 0.0001$).

Table 2. Pre-operative and Post-operative ODI and VAS Score Results.

		Mean (95% CI)	**SD**
ODI%	Pre	46.4 (42.6, 50.2)	17.3
	Post	9.3 (7.5, 11.0)	7.6
Back VAS	Pre	8.0 (7.3, 8.7)	2.8
	Post	3.5 (3.0, 4.1)	2.3
Leg VAS	Pre	8.2 (7.6, 8.8)	2.5
	Post	2.9 (2.3, 3.6)	2.6

This table presents the mean scores and standard deviations for the Oswestry Disability Index (ODI) and Visual Analogue Scale (VAS) both before and after the surgical intervention. Scores are meant to illustrate the effectiveness of the procedure in improving patient-reported outcomes

Table 3. Statistical Analysis of ODI and VAS Score Improvements.

	Mean difference (95% CI)	**SD**	**Paired t (p)**
ODI%	-37.1 (-41.2, - 33.1)	17.3	-18.26 (<0.001)
Back	-4.5 (-5.1, -3.9)	2.4	-15.88 (<0.001)
Leg	-5.3 (-5.9, -4.6)	2.9	-15.49 (<0.001)

This table details the statistical analysis performed using paired samples t-tests to compare pre-operative and post-operative changes in ODI and VAS scores. The mean differences, 95% confidence intervals (CI), and p-values are provided to quantify and validate the significance of the observed improvements.

4. Discussion

Spinal disorders in the ever-growing elderly population are quite common and can present with debilitating symptoms. Although patients may respond to conservative management, we know from the SPORT trial data that surgical management of lumbar spinal stenosis and degenerative spondylolisthesis has better long-term results. Therefore, it is increasingly important to identify safe and effective interventions that improve the quality of life of elderly patients with such disorders while simultaneously lowering costs for healthcare. Spine surgery is usually indicated only after nonsurgical treatments (pain medications, pain management blocks, and physical therapy) have failed to relieve severe symptoms. Most spine surgery complications are related to excessive blood loss necessitating a blood transfusion, wound healing issues, infections, cerebrospinal fluid leakage, hardware misplacement, pulmonary embolism, and thrombophlebitis [9-11].

Minimally invasive transforaminal lumbar interbody fusion (MIS TLIF) has become a widely adopted surgical approach in treating degenerative lumbar diseases. Minimally invasive spine surgery (MIS), as an approach to the spine, is associated with less intra-operative blood loss, less muscle tissue dissection, and a shorter length of stay when compared to open lumbar fusions. MIS also translates to cost savings for hospitals and the healthcare system due to shorter length of stay and decreased inpatient resource utilization (such as inpatient rehabilitation programs and narcotic medication overuse). Literature reviews on perioperative outcomes between MIS TLIF and open TLIF have reported significantly better perioperative outcomes, at least in the short-term, in MIS TLIF compared to open TLIF [11,12]. It is important to note that MIS surgery may be cumbersome for pedicle screw placement accuracy. To increase the reproducibility of acquiring good to excellent screw placement trajectories, and to improve patient safety, we introduced robotic spine surgery with the Medtronics Mazor X robot (Medtronics Sofamor Danek, Memphis, TN) back in 2018, and it has just recently been updated to the navigated stealth edition.

The present study examined the clinical outcomes for patients 65 and over who underwent MIS-TLIF for lumbar spinal stenosis with or without degenerative lumbar spondylolisthesis in one or two lumbar levels. Previous studies that have examined retrospective data comparing MIS and open TLIF approaches have found lower blood loss and shorter hospital length of stay in MIS TLIF compared to open TLIF [5,8]. In our study, the mean estimated blood loss (78.8 mL) and length of hospitalization (1.1 days) were significantly lower when compared to open TLIF reported outcomes in other studies. For instance, Villavicencio et al. reported a greater mean EBL (366.8 mL) and length of hospitalization (4.2 days) in their open TLIF group [8]. Additionally, Dhall et al. also reported a greater mean EBL (505 mL) and length of hospitalization (5.5 days) in their open TLIF group [5].

Most patients visit surgeons' clinics with the desire to reduce pain and regain some of their functionality. Consequently, MIS TLIF has been found to provide patients with a significant reduction in back and leg pain [12, 13]. Moreover, another retrospective study of 40 patients who underwent MIS TLIF for spondylolisthesis also found a significant reduction in back and radicular leg pain from VAS 52 and 65 to 15 and 8 [7]. Still, Lee et al. reported a statistically significant reduction in back pain VAS scores for the MIS group compared to the open group at postoperative six months and one-year marks ($P < 0.05$) [12]. In our study, we observed a significant reduction in back and radicular leg pain from the preoperative baseline to the one-year mark. The mean preoperative back and radicular pain VAS scores were 8.0 and 8.2 respectively. Postoperative year one, the mean VAS pain scores reduced to 3.5 for back pain (mean difference -4.5; 95% CI -5.1 to -3.9; p-value <0.0001), and 2.9 for radicular leg pain (mean difference -5.2; 95% CI -5.9 to -4.6; p-value <0.0001). These results were considered a clinically and statistically significant improvement in VAS pain scores for our patients.

Most of these patients seeking a spine surgery consultation have lost some degree of function secondary to pain, limited range of motion, and nerve compression. In our study, functional outcome was measured using the ODI questionnaire. Previous prospective studies have shown a significant reduction in mean ODI scores in patients who underwent MIS TLIF [12]. Deutsch and Musacchio found that 85% of their cases had a greater than 20-point reduction postoperatively in ODI scores after an MIS TLIF [14]. One open surgery technique study showed mean ODI scores for the MIS group dropped 25.5 points, from 51 pre-operatively to 25.5 at the one-year post-op mark, while the open group went from a pre-op ODI score of 55.2 to 36.4 at the one-year post-op mark (18-point drop). Statistical analysis would show significance in these ODI differences with a p-value of < 0.05 [12]. In this study, we demonstrated similar results since our mean preoperative and postoperative ODI percentage scores were 46.4% and 9.3%, respectively. This means that our patients also had a greater than 20-point reduction in their ODI scores after an MIS TLIF. Statistical analysis yielded an ODI percentage score mean difference of -37.1, with 95% CI -41.2 to -33.1, and a p-value of <0.0001.

Limitations to this study include its retrospective nature and short one- to two-year follow-up data in most cases. In addition, all cases are from a single surgeon that only performs MIS robotic TLIFs and has no other comparable open cases. Therefore, our comparison data comes from published literature on open lumbar fusion cases. Future papers should attempt prospective randomized, double-blinded studies to weigh in on the unanswered questions more heavily and explore outcomes on populations with diverse ethnic backgrounds. Specifically, whether patients 65 and over undergoing a one or two-level fusion should be operated via a long-held traditional open approach or benefit more from an MIS approach to the spine, which preserves what little muscle capacity they have left.

5. Conclusions

Our retrospective cohort analysis suggests a potential association between robotically assisted MIS TLIFs and statistically significant reductions in both VAS and ODI scores when comparing preoperative baselines to one-year postoperative outcomes. These findings could indicate improvements in axial low back and radicular leg pain, as well as enhanced functionality and daily living activities—two key factors patients often consider when evaluating surgical options. While these results align with previous studies on minimally invasive techniques in younger populations, we now observe similar outcomes in a Hispanic population aged 65 and older. Given the growing aging population and the potential advantages MIS may offer over open surgery in the short term, it would seem appropriate to consider MIS techniques more routinely for older adults, particularly when one or two levels are involved. Older, physically vulnerable patients may benefit from the muscle-sparing nature of MIS procedures. Additionally, the literature suggests that MIS may lead to reduced blood loss, lower tissue dissection, and limited subperiosteal stripping, potentially resulting in faster ambulation, reduced inpatient resource use (such as physical therapy and narcotics), lower infection rates, and shorter hospital stays.

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Original Article

Biomarkers for Prostate Cancer Aggressiveness in Puerto Rican Men: Analysis of Phospho-Rb S249, N-cadherin, β-catenin, and E-cadherin Expression in Prostate Biopsies

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Abstract: Prostate cancer (PCa) is the leading cause of cancer in Puerto Rican men and exhibits significant racial disparities globally. Although only 8% of cases invade beyond the prostate, predicting PCa aggressiveness is challenging. This study investigated the potential of the retinoblastoma tumor suppressor protein phosphorylated in Serine 249 (Phospho-Rb S249), N-cadherin, β-catenin, and E-cadherin as biomarkers for identifying aggressive PCa in Puerto Rican men. We hypothesized that the expression of these proteins could serve as biomarkers for identifying PCa tumors with potential to becoming aggressive in Puerto Rican men. Immunohistochemistry was performed on 23 biopsies from Puerto Rican men to evaluate the biomarkers' expression, and correlation analyses examined associations with clinicopathological parameters. Results showed that Phospho-Rb S249 expression correlated positively with tumor size and positive cores in patients with Gleason scores ≥4+3. β-catenin was positively associated with tumor size and carcinoma percentage in Gleason scores ≥4+3. E-cadherin expression negatively correlated with grade group, indicating a protective role. In contrast, N-cadherin and β-catenin were more prominent in Gleason scores ≤3+4, hinting at their involvement in early epithelial-to-mesenchymal transition (EMT). A decision tree analysis identified N-cadherin expression as a key determinant for classifying PCa aggressiveness, with an 82% likelihood. These findings suggest N-cadherin as a biomarker for identifying PCa with the potential to become aggressive. While our study provides promising results, further validation in a larger patient cohort is needed to increase the robustness and reliability of our findings. Also, combining multiple biomarkers could further enhance the specificity of aggressive PCa detection.

Keywords: Prostate cancer, β-catenin, Puerto Rico, Epithelial-to-Mesenchymal Transition (EMT)

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1. Introduction

Prostate cancer (PCa) accounted for 29% of all cancer diagnoses in 2023, and since 2010, there has been an increase in cases diagnosed at higher grades and advanced stages [1,2]. For PCa to metastasize, cancer cells must acquire a

advanced stages [1,2]. For PCa to metastasize, cancer cells must acquire a migratory and invasive phenotype [3]. However, only about 8% of cases invade beyond the prostate to other body parts like the bones or lymph nodes [4]. Thus, overtreatment is a common problem in PCa screening, ranging from 1.7% to 67% of cases [5]. PCa overtreatment leads to long-term urinary, erectile, and bowel dysfunction in prostatectomy and radiation patients, impacting the patient's quality of life and healthcare costs [6]. Thus, prognostic tests, including gene panels like Prolaris and Oncotype DX, have been developed to help clinicians identify PCa with aggressive potential and guide disease management by predicting the likelihood of progression [7]. However, evidence supporting their clinical utility is limited. Prolaris showed no significant impact on treatment plans or patient outcomes, while Oncotype DX had mixed effects on physician treatment recommendations [8,9,10]. Moreover, the effectiveness of Oncotype DX is diminished in African American men because the test mainly relies on data from Caucasian populations, limiting its relevance for other ethnic groups [11].

Minorities, such as Black and Hispanic men, are underrepresented in PCa clinical trials, making it challenging to manage their disease [12]. Compared to US-Hispanics and non-Hispanic whites, Puerto Ricans have a 40% higher PCa mortality rate, associated with low socioeconomic status [13]. Additionally, studies evaluating Hispanic/Latino populations have found that Puerto Ricans experience higher (HR=1.70, 95% CI 1.28 to 2.25, $p<0.001$) PCa-specific mortality compared to other Hispanic/Latino groups [14]. This heterogeneity affects clinical practice, as minority groups are often categorized in US or Hispanic data, which may not reflect the true impact of the disease in these populations [15]. Therefore, we cannot be certain whether existing PCa prognostic tests will be equally effective in Puerto Ricans.

Cadherin and catenins are crucial for epithelial cell adhesion, and their disruption can promote tumor progression [16]. E-cadherin has been shown to play a crucial role in maintaining the integrity of adherens junctions and epithelial organization [17]. During embryonic development, E-cadherin is crucial for tissue formation and cell rearrangement, while its reduced expression in adult tissues is associated with loss of contact inhibition, increased cell motility, and advanced cancer stages [18,19]. β-Catenin interacts with other catenins and plays an anti-oncogenic role when localized at the cell membrane [20]. However, when overexpressed in the nucleus of cells, it becomes oncogenic and promotes invasive PCa [20]. The reduced expression or functional loss of E-cadherin and its complex with β-catenin is considered a hallmark of epithelial-to-mesenchymal transition (EMT), a process in which epithelial cells change into mesenchymal cells, leading to cancer spread [21,22]. During EMT, a cadherin switch from E-cadherin to N-cadherin occurs, which strongly suggests PCa progression and poor prognosis [23]. This emphasizes the importance of E-cadherin, N-cadherin, and β-catenin as potential biomarkers for detecting aggressive PCa and monitoring disease progression.

We studied the Retinoblastoma (Rb) protein, a critical regulator of the cell cycle, for its potential in the early detection of aggressive PCa in Puerto Rican men. Loss of Rb's tumor suppressive function can induce cell cycle deregulation and lead to a malignant phenotype in various cancers [24]. Post-translational modifications, such as phosphorylation, on Rb play a crucial role in modulating its function and cancer progression [25]. In PCa, Rb loss occurs during disease progression, particularly as tumors become resistant to castration treatments [26]. However, there is a lack of studies examining the functional role of Rb phosphorylation sites. In our previous research on lung cancer, we found a positive correlation between Phospho-Rb S249 levels and tumor grade, indicating a potential link between this specific phosphorylation and tumor aggressiveness [27].

In a previous PCa study conducted in our laboratory, these biomarkers were assessed in an Asian cohort. The findings indicated that in Asian patients, there is

a negative correlation between the expression of E-cadherin and β-catenin with aggressive tumor behavior, whereas Phospho-Rb S249 and N-cadherin were positively correlated with increased tumor aggressiveness [28]. Furthermore, when Asian patients were stratified based on Gleason scores to assess the ability of the biomarkers to identify aggressive PCa, β-catenin emerged as a key classifier for distinguishing between low- and high-risk diseases [28]. Thus, in this study, we assessed the potential of Phospho-Rb S249, N-cadherin, β-catenin, and E-cadherin as biomarkers for identifying PCa tumors with a high risk of becoming aggressive among Puerto Rican men. We hypothesized that the expression levels of these biomarkers could help distinguish PCa tumors with aggressive potential in this population. Our results showed that in patients with Gleason score ≤3+4, E-cadherin negatively correlated with tumor's grade group. In contrast, in patients with Gleason scores ≥4+3, Phospho-Rb S249 was positively correlated with tumor size and the total number of positive cores. Additionally, β-catenin was positively correlated with tumor size and the percentage of carcinoma in the tissue, while E-cadherin was negatively correlated with tumor's grade group. Also, N-cadherin and β-catenin were more prominent in patients with Gleason scores ≤3+4, potentially due to their involvement in early EMT. Finally, a classification tree revealed that N-cadherin expression is a critical biomarker for identifying Puerto Rican PCa patients with a high potential for aggressive disease, indicating that this biomarker may have practical utility in clinical settings.

2. Methodology

2.1 Puerto Rican PCa biopsy samples

Puerto Rican PCa biopsy samples were obtained from "CorePlus Servicios Clínicos y Patológicos" in Carolina, Puerto Rico. In this study, all patient biopsy samples were retrospectively collected; we did not perform any patient recruitment. Thus, we received an Institutional Review Board (IRB) exemption with protocol number 2207109755. The samples provided by "CorePlus Servicios Clínicos y Patológicos" were already prepared as formalin-fixed and paraffin-embedded on a positively charged glass slide. Each slide contained a single patient biopsy sample, with each tumor section measuring 5μm in thickness. Also, "CorePlus Servicios Clínicos y Patológicos" provided the demographic and pathological information for each patient. The information included the patient's age, PSA levels (ng/ml), tumor size (millimeters), grade group, Gleason score, percentage of the biopsy with carcinoma, and the number of positive cores for each patient. The control group consisted of prostate biopsy samples selected based on the absence of carcinoma, classified as negative following standard pathological evaluation. In total, 23 PCa adenocarcinoma cases and nine prostate control cases were analyzed in the study.

2.2 Immunohistochemistry (IHC)

Immunohistochemistry (IHC) was performed to assess the expression of Phospho-Rb S249, N-cadherin, β-catenin, and E-cadherin in the PCa biopsy samples, following a previously established protocol [29]. Paraffin was first removed from the slides, and the samples were hydrated and treated to block endogenous peroxidase activity. Antigen retrieval was then carried out, followed by incubation with primary and secondary antibodies. The antibodies used in this study were Phospho-Rb S249 (Abcam, Cat. No. ab4788) 1:100, purified mouse anti-N-cadherin (BD Biosciences, Cat. No.610920) 1:125; β-catenin (Cell Signaling, Cat. No. 8480S) 1:200; and E-cadherin (Cell Signaling, Cat. No. 3195S) 1:400. The secondary antibody used was the Super Sensitive Link Label IHC kit (BioGenex, Cat. No. LP000-ULE). All samples were exposed to one drop of Diaminobenzidine (DAB) (BioGenex, Cat. No. HK542-XAKE) for one minute and 30 seconds. Then, slides are dehydrated, cleared in xylene, and mounted with coverslips, to be analyzed under a light microscope. Slides were preserved with a coverslip and Cytoseal 60 (Thermo Scientific, Cat. No. 8310-16) to protect the samples and ensure optimal examination on the microscope for

the image acquisition. The image acquisition was performed using the NIS-Element AR software and the Olympus BX60 microscope. The expression of N-cadherin, β-catenin, and E-cadherin was assessed based on their membranous localization, while Phospho-Rb S249 expression was evaluated in the nucleus. This is because N-cadherin, β-catenin, and E-cadherin function as epithelial markers, whereas when Rb gets phosphorylated and inactivated, it exhibits nuclear localization. All tissue cores were analyzed in four quadrants at 40X magnification. Immuno-stained slides were independently and blindly scored for the number of immune-positive cells in each biopsy. We assigned scores using objective measures with the Image J program, which automatically provided a scale of positivity after selecting a manual threshold of 37%. This scale was categorized as negative, low positive, positive, or high positive. We translated this scale into a numerical format ranging from 1 to 4, with 1 indicating a negative result and 2, 3, and 4 representing low positive, positive, and high positive results, respectively.

2.3 Statistical Analysis

Graph Pad Prism software version 9 was used to describe the sample population using measures of central tendency and dispersion (mean, standard deviation, median, 25th and 75th percentiles) for continuous variable; categorical variables were described with frequencies and percentages. A Spearman correlation analysis was performed to assess the strength and direction of the relationship between the biomarker's expression and the clinicopathologic data. Patients were stratified into two groups based on their Gleason score, following the National Comprehensive Cancer Network (NCCN) guidelines (Table 1) [30]. One group consisted of patients with a Gleason score pattern equal to or less than 3+4, whereas the second group consisted of patients with a Gleason score pattern equal to or higher than 4+3. The Wilcoxon rank-sum test was employed to perform group comparisons after stratifying patients based on their Gleason score. This analysis was performed using STATA version 17 (STATA Corp, College Station, TX, USA). Lastly, R Studio version 4.3.0 was used to construct a classification tree aimed at assessing whether the expression of the biomarkers could identify patients at risk of developing aggressive PCa. A split ratio of 0.70 was employed to allocate a larger portion of the dataset to the training set, enabling better tree construction. Additionally, parameters were set with a minimum split of five and a complexity parameter (cp) value of 0.01 to control tree depth and complexity. We also assessed the performance of the predictive model by analyzing its Receiver Operating Characteristic (ROC) curve. A significance level of $p \leq 0.05$ was established to be statistically significant for all analyses.

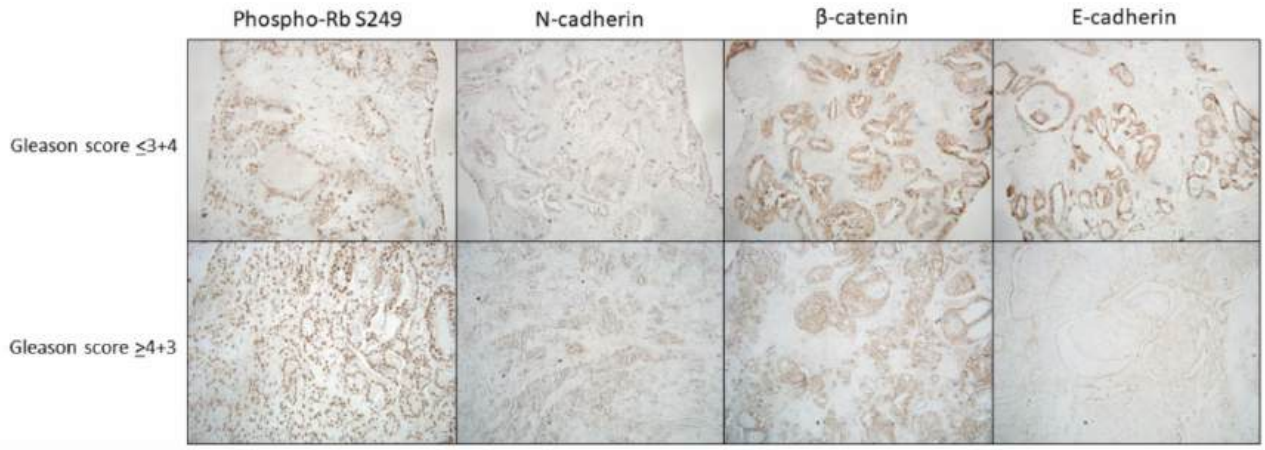
Table 1. Description of PCa tissue characteristics based on Gleason score patterns as defined by the NCCN guidelines.

Gleason patterns	Gleason score	Description
≤3+3	≤6	Well-formed glands
3+4	7	Predominantly well-formed glands with lesser poorly formed/fused/cribriform glands
4+3	7	Predominantly poorly formed/fused/cribriform glands with lesser component of well-formed glands
4+4, 3+5, 5+3	8	Only poorly formed/fused/cribriform glands
4+5, 5+4, 5+5	9 or 10	Lack gland formation (or with necrosis) with or without poorly formed/fused/cribriform glands

3. Results

3.1 Biomarker expression and Gleason score stratification in Puerto Rican PCa patients

To investigate the potential of Phospho-Rb S249, N-cadherin, β-catenin, and E-cadherin as biomarkers for identifying aggressive PCa in Puerto Rican patients, we stratified the patients based on their Gleason score (N=23), a key factor in determining the prognosis of prostatic malignancies [31]. PCa patients with a Gleason score of 4+3 or higher have worse overall survival, cancer-specific survival, and increased risk of progression compared to those with Gleason score 3+4 or lower [32,33]. Accordingly, one group included patients with Gleason scores of 3+4 or lower (N=8), and the other included patients with Gleason scores of 4+3 or higher (N=15). Figure 1 illustrates the IHC staining results for the expression of the biomarkers (Phospho-Rb S249, N-cadherin, β-catenin, and E-cadherin) stratified by Gleason score categories (≤3+4 vs. ≥4+3) based on their mean expression levels. Nuclear expression of Phospho-Rb S249 was not significantly different between the groups (*p*-value: 0.06250). N-cadherin expression was slightly higher in patients with Gleason scores ≤3+4 compared to those with scores ≥4+3 (*p*-value: 0.0547). In contrast, β-catenin and E-cadherin expression levels were slightly higher in the ≤3+4 group than in the ≥4+3 group, with *p*-values of 0.0859 and 0.5625, respectively. However, no significant differences were observed in the expression levels of these biomarkers between the Gleason score categories.



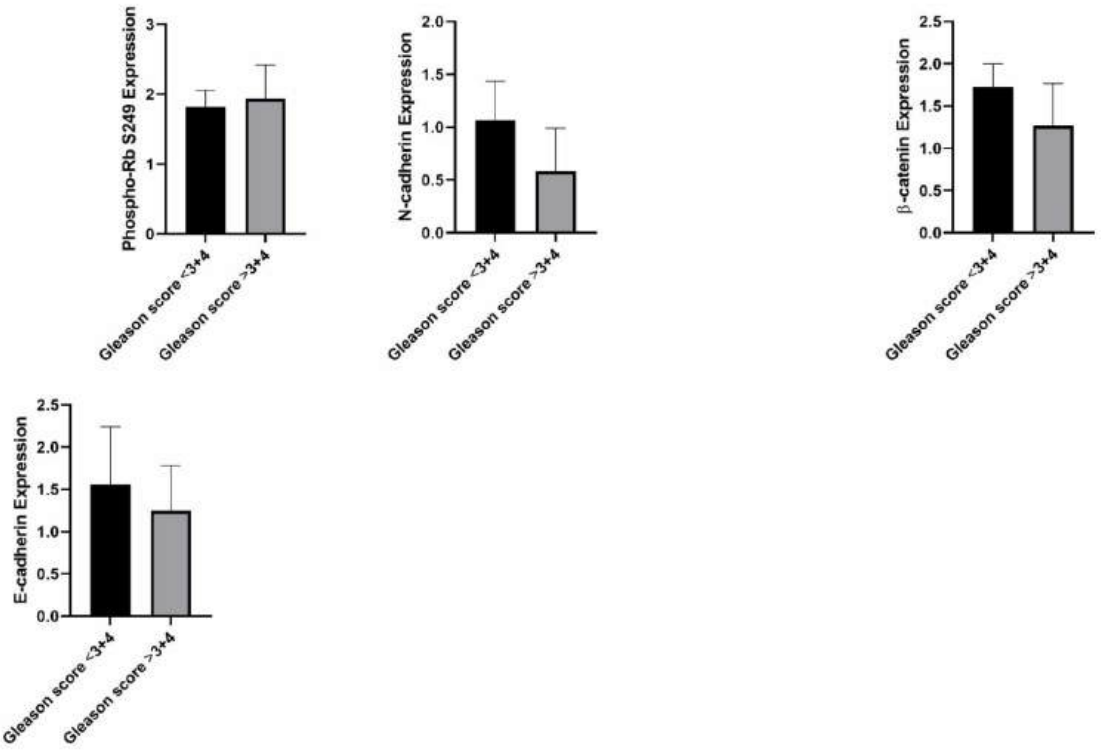


Figure 1. IHC staining of the biomarkers Phospho-Rb S249, N-cadherin, β-catenin, and E-cadherin. The upper section of the figure corresponds to a patient with a Gleason score of 3+3, representing the staining patterns that predominate within the Gleason scores ≤3+4, a less aggressive form of PCa. In the lower section of the figure, the staining corresponds to a patient with Gleason score of 4+5, representing the pattern that predominates in patients with Gleason scores ≥4+3, a more aggressive form of PCa. Pictures were captured at 40X visual fields. Bars represent mean expression levels, and error bars indicate ± standard deviation (SD).

Table 2 presents the frequency and percentage of clinicopathological parameters based on Gleason score. No differences were observed in age between the groups (p-value>0.05). The median tumor size was larger in patients with Gleason score ≥4+3 (6.75 mm vs. 5.25 mm), although this difference was not statistically significant (p-value>0.05). Similarly, patients with Gleason scores ≥4+3 had a higher median percentage of carcinoma in their biopsies (34.0% vs. 30.5%) and a greater median number of positive cores (6.0 vs. 2.0), with the latter nearing statistical significance (p-value: 0.054). We further examined how Phospho-Rb S249, N-cadherin, β-catenin, and E-cadherin were expressed across the Gleason scores categories based on the median (Table 3). N-cadherin expression was higher in patients with Gleason scores ≤3+4, with a median of 1.12 compared to 0.75 in those with Gleason scores ≥4+3 (p-value: 0.023). Also, β-catenin showed statistical significance in patients with Gleason scores ≤3+4 (median:1.75 vs. 1.25 for Gleason scores ≥4+3, p-value: 0.018). However, there were no significant differences in the expression of Phospho-Rb S249 or E-cadherin between the two Gleason score categories in this cohort (p-value>0.05).

Table 2. Clinical profile of the Puerto Rican PCa patients after stratifying them by their Gleason scores. Table shows the number of frequency and percentage of contribution in each category as "N(%)".

Variable	Overall (N=23)	Gleason score ≤ 3 + 4 (N=8)	Gleason score ≥ 4 + 3 (N=15)	*p*-value
Age (years)				0.581
Mean (sd)	69.65 (8.10)	67.75 (9.29)	70.67 (7.54)	
Median (p25, p75)	70.0 (66.0, 75.0)	71.5 (64.0, 73.5)	70.0 (66.0, 75.0)	
Gleason score				-
6	3 (13.04)	3 (37.50)	0	
7	11 (47.83)	5 (62.50)	6 (40.00)	
8	4 (17.39)	0	4 (26.67)	
9	4 (17.39)	0	4 (26.67)	
10	1 (4.35)	0	1 (6.67)	
Grade group				-
1	3 (13.04)	3 (37.50)	0	
2	5 (21.74)	4 (50.00)	1 (6.67)	
3	5 (21.74)	0	5 (33.33)	
4	4 (17.39)	0	4 (26.67)	
5	6 (26.09)	1 (12.50)	5 (33.33)	
Tumor size (N=22)				0.142*
Mean (sd)	7.54 (5.60)	4.78 (1.95)	9.11 (6.42)	
Median (p25, p75)	5.8 (4.2, 9.0)	5.25 (3.65, 5.9)	6.75 (4.50, 14.40)	
Percentage with carcinoma				0.478*
Mean (sd)	42.65 (29.08)	36.3 (27.71)	46.03 (30.16)	
Median (p25, p75)	34.0 (20.0, 58.0)	30.5 (12.65, 56.5)	34.0 (22.0, 72.0)	
Number of positive cores				0.054*
Mean (sd)	5.70 (3.55)	4.00 (3.59)	6.60 (3.29)	
Median (p25, p75)	5.0 (2.0, 8.0)	2.0 (2.0, 6.0)	6.0 (4.0, 8.0)	
PSA (N=21)				<0.001*
Mean (sd)	28.81 (38.90)	5.82 (1.04)	40.30 (43.60)	
Median (p25, p75)	11.51 (5.93, 18.74)	5.80 (5.07, 6.62)	16.96 (11.51, 62.25)	

sd: standard deviation, p25 or 75: 25- & 75-percentile, *Wilcoxon rank test

Table 3. Puerto Rican PCa biomarker's expression after stratifying them by their Gleason scores. Table shows the number of frequency and percentage of contribution in each category as "N(%)".

Variable	Overall (N=23)	Gleason score ≤ 3 + 4 (N=8)	Gleason score ≥ 4 + 3 (N=15)	*p*-value*
Phospho-Rb S249				0.570
Mean (sd)	1.95 (0.47)	1.97 (0.47)	1.93 (0.49)	
Median (p25, p75)	2.0 (1.75, 2.0)	1.75 (1.75, 2.13)	2.0 (1.75, 2.0)	
N-Cadherin				0.023
Mean (sd)	0.75 (0.45)	1.06 (0.37)	0.58 (0.41)	
Median (p25, p75)	0.75 (0.25, 1.0)	1.12 (0.75, 1.37)	0.75 (0.25, 1.0)	
E-cadherin				0.191
Mean (sd)	1.36 (0.59)	1.56 (0.68)	1.25 (0.53)	
Median (p25, p75)	1.5 (1.0, 2.0)	1.25 (2.12)	1.0 (0.75, 1.75)	
***β*-Catenin**				0.018
Mean (sd)	1.42 (0.48)	1.72 (0.28)	1.27 (0.50)	
Median (p25, p75)	1.5 (1.25, 1.75)	1.75 (1.5, 2.0)	1.25 (1.0, 1.5)	

sd: standard deviation, p25 or 75: 25- & 75-percentile, *Wilcoxon rank test

3.2 Correlation analysis between biomarker expression and clinicopathological parameters

We conducted a correlation analysis to explore the relationships between the expression of Phospho-Rb S249, N-cadherin, β-catenin, and E-cadherin, and various clinical parameters, including age, PSA, grade group, tumor size, percentage of carcinoma, and the number of positive biopsy cores (Tables 4 and 5). In patients with Gleason scores ≤3+4, we observed a significant negative correlation between E-cadherin expression and the grade group (r: -0.759, *p*-value: 0.029), a prognostic scale ranging from one to five linked to biochemical recurrence and prostate-specific mortality [34]. However, no significant correlations were found for Phospho-Rb S249, N-cadherin, or β-catenin with any clinical parameter in this category. In contrast, Phospho-Rb S249 expression was positively correlated with tumor size (r: 0.616, *p*-value: 0.019) and the number of positive biopsy cores (r: 0.522, *p*-value: 0.046). Similarly, β-catenin expression showed positive correlations with tumor size (r: 0.570, *p*-value: 0.033) and the percentage of carcinoma in the biopsy samples (r: 0.635, *p*-value: 0.011). Additionally, E-cadherin negatively correlated with the grade group (r: -0.566, *p*-value: 0.028). No significant correlations were identified between N-cadherin expression and any clinicopathological parameters in patients with Gleason scores ≥4+3.

Table 4. Correlation of Phospho-Rb S249, N-cadherin, β-catenin, and E-cadherin expression with the clinicopathological data of patients with Gleason scores ≤3+4.

Variables	r*	*p*-value
Phospho-Rb S249		
Age (years)	0.340	0.410
PSA (ng/ml) (n=7)	0.394	0.382
Grade group	0.125	0.768
Tumor size (mm)	-0.355	0.388
% with carcinoma	-0.051	0.905
Number of positive cores	0.155	0.713
N-cadherin		
Age (years)	-0.221	0.599
PSA (ng/ml) (n=7)	-0.367	0.418
Grade group	-0.259	0.536
Tumor size (mm)	0.412	0.310
% with carcinoma	0.449	0.265
Number of positive cores	0.245	0.558
***β*-catenin**		
Age (years)	0.306	0.461
PSA (ng/ml) (n=7)	-0.386	0.393
Grade group	-0.609	0.109
Tumor size (mm)	-0.037	0.931
% with carcinoma	-0.321	0.438
Number of positive cores	-0.421	0.299
E-cadherin		
Age (years)	-0.500	0.207
PSA (ng/ml) (n=7)	-0.273	0.554
Grade group	-0.759	0.029
Tumor size (mm)	-0.108	0.798
% with carcinoma	-0.241	0.565
Number of positive cores	-0.449	0.264

*Spearman correlation

Table 5. Correlation of Phospho-Rb S249, N-cadherin, β-catenin, and E-cadherin expression with the clinicopathological data in patients with Gleason scores ≥4+3.

Variables	r*	*p*-value
Phospho-Rb S249		
Age (years)	-0.300	0.278
PSA (ng/ml) (N=14)	0.120	0.683
Grade group	0.285	0.302
Tumor size (mm) (N=14)	0.616	0.019
% with carcinoma	0.394	0.147
Number of positive cores	0.522	0.046
N-cadherin		
Age (years)	0.429	0.111
PSA (ng/ml) (N=14)	0.340	0.234
Grade group	0.061	0.829
Tumor size (mm) (N=14)	-0.208	0.475
% with carcinoma	0.124	0.660
Number of positive cores	0.082	0.771
β-catenin		
Age (years)	0.139	0.620
PSA (ng/ml) (N=14)	0.013	0.963
Grade group	-0.069	0.808
Tumor size (mm) (N=14)	0.570	0.033
% with carcinoma	0.635	0.011
Number of positive cores	0. 383	0.158
E-cadherin		
Age (years)	-0.340	0.215
PSA (ng/ml) (N=14)	-0.357	0.210
Grade group	-0.566	0.028
Tumor size (mm) (N=14)	0.126	0.668
% with carcinoma	0.216	0.439
Number of positive cores	-0.182	0.516

*Spearman correlation

3.3 Classification tree analysis for aggressive PCa identification

To evaluate whether the expression of the biomarkers Phospho-Rb S249, N-cadherin, β-catenin, and E-cadherin could identify patients at risk of developing an aggressive PCa, a classification tree was constructed (Figure 2). This analysis identified that N-cadherin expression is a critical determinant for classifying the PCa aggressiveness in this dataset. Specifically, patients with N-cadherin expression levels ≥1.1 (low positive or higher) have an 18% probability of having Gleason scores ≤3+4, with a predicted likelihood of 0% (0.00) belonging to the Gleason score ≥4+3 category. Conversely, PCa patients with N-cadherin expression levels <1.1 (negative expression) were classified as having Gleason scores ≥4+3, with an 82% probability and a 79% (0.79) predicted likelihood of belonging to it. The classification tree demonstrated an AUC of 0.75.

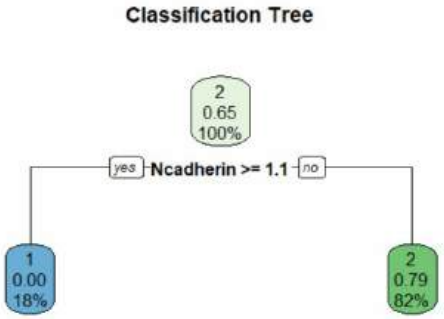


Figure 2. Classification tree for predicting PCa aggressiveness based on N-cadherin expression levels.

4. Discussion

This study aimed to evaluate the potential of Phospho-Rb S249, N-cadherin, β-catenin, and E-cadherin as biomarkers for identifying PCa with high risk of becoming aggressive in Puerto Rican men. For this, we explored the relationship between the expression of Phospho-Rb S249, N-cadherin, β-catenin, and E-cadherin with the clinicopathological parameters of the Puerto Rican PCa patients. When evaluating the clinicopathological parameters, we observed an overall trend of increasing tumor aggressiveness in patients with higher Gleason scores. Furthermore, the evaluation of the biomarker's expression levels showed that N-cadherin and β-catenin were highly expressed in Gleason scores ≤3+4. These results potentially reflect the early onset of EMT, contributing to disease progression.

N-cadherin expression has been noted in multiple human cancers, such as those of the breast, lung, and liver, among others [35,36,37]. This occurs in some cases irrespective of E-cadherin status [38,39]. The appearance of N-cadherin expression is among the earliest events in malignant transformation. Also, low N-cadherin expression has been found in cervical lesions and neoplasia, that have precancerous potential [40,41]. Additionally, the hallmark of EMT starts with the upregulation of N-cadherin, accompanied by the downregulation of E-cadherin, a process regulated by a complex network of signaling pathways and transcription factors [42]. Consequently, the presence of N-cadherin in the early stages of cancer may stimulate cell migration and invasion, contributing to tumorigenesis [43].

The expression pattern of N-cadherin observed in this Puerto Rican cohort suggests that lower Gleason score tumors might exhibit elevated N-cadherin levels, possibly indicating a role in early tumor characteristics rather than advanced disease progression. Additionally, the high levels of β-catenin in patients with Gleason scores ≤3+4 emphasize its role in maintaining cell-cell adhesion during the initial stages of the disease. The interaction between β-catenin and E-cadherin at the membrane may prevent β-catenin from translocating to the nucleus and participating in the transcriptional activation of factors such as LEF-1/TCF [44]. Conversely, studies have shown that Wnt signaling activation can increase β-catenin levels in the membrane, enhancing its recruitment to existing adherens junctions [45]. Thus, the presence of N-cadherin and β-catenin in tumors with Gleason scores ≤3+4 may suggest an early transition toward more aggressive disease. Furthermore, our findings indicated that reduced E-cadherin expression was significantly associated with the grade group in patients with both Gleason scores ≤3+4 and ≥4+3. This association suggests that E-cadherin downregulation is closely related to more

aggressive and poorly differentiated tumors. Consequently, decreased E-cadherin expression may be indicative of more aggressive disease in high-risk patients. Our findings align with previous studies that emphasize the role of E-cadherin dysfunction or loss of expression in cancer progression, as it leads to reduced cellular adhesion in epithelial tissues [18].

Moreover, we observed correlations between increased β-catenin expression with the percentage of carcinoma in biopsies and tumor size in patients with Gleason scores ≥4+3. It is well established that E-cadherin inhibits the nuclear localization and transactivation of β-catenin [46]. Therefore, the loss of E-cadherin's growth suppressor activity disrupts this inhibition, allowing β-catenin to translocate into the nucleus, where it can bind to TCF/LEF and activate the Wnt/β-catenin signaling pathway, promoting tumorigenesis [47]. This finding is consistent with the established role of β-catenin in cancer progression, as its nuclear translocation is often associated with increased invasiveness and metastasis in various cancers [48,49,50]. In vitro studies have also confirmed that the disruption of β-catenin and E-cadherin leads to a loss of intercellular adhesion and enhanced tumor invasion in human cancer cells [51]. Consequently, our findings of increased β-catenin expression in patients with Gleason scores ≥4+3 may reflect the nuclear translocation of this protein and the activation of the Wnt/β-catenin signaling pathway. Importantly, while N-cadherin did not show significant correlations with the clinicopathological parameters in this cohort of Puerto Rican PCa patients, its role remains relevant, especially in the context of EMT.

We also assessed the potential of Phospho-Rb S249 as a biomarker for identifying PCa with potential to become aggressive. The Rb protein contains 14 mono-phosphorylated sites, each potentially influencing Rb interactions and conferring functional specificity [52]. Rb is known to be inactivated through hyper-phosphorylation, and distinct phosphorylation patterns are observed in different cancer types, suggesting that phosphorylation-based mechanisms may drive Rb dysfunction in a cancer-specific manner [53]. Thus, it has been suggested that Rb protein may have a phosphorylation code that controls specific activities in various processes regulating proliferation [53]. However, the role of Rb S249 has only been studied in conjunction with threonine 252 (T252) in previous research [54,55]. Notably, no clear association has been established between specific Rb phosphorylation events and PCa progression. While limited research exists on Phospho-Rb S249 in cancer, our previous study on lung cancer found that combined expression of Phospho-Rb S249 with p39 and E-cadherin was associated with advanced tumor staging in non-small cell lung carcinoma [27]. Here, we have identified that increasing levels of Phospho-Rb S249 correlated with tumor size and number of positive cores in Puerto Rican patients with Gleason scores ≥4+3, revealing a potential role in aggressive PCa. However, Phospho-Rb S249 has a role during early G1, where cyclin D/Cdk4/6 phosphorylates Rb at residues S249, T356, S807, S811, and T826, whereas in late G1 and early S, cyclin E/Cdk2 phosphorylates Rb at residues S612 and T821 [56]. Therefore, subsequent phosphorylation events, such as those at S612 or T821, in conjunction with S249 may provide better predictive value for identifying PCa patients at risk of progressing to aggressive disease; however, this hypothesis requires further investigation.

Comparing our findings with those of our previous study on Asian PCa patients, both studies demonstrated that Phospho-Rb S249, N-cadherin, β-catenin, and E-cadherin were significantly associated with tumor aggressiveness [28]. However, differences in biomarker behavior were observed between the populations. In the Asian cohort, β-catenin emerged as the primary classifier for aggressive PCa, whereas in the Puerto Rican cohort, N-cadherin was the most informative biomarker for distinguishing aggressive PCa. These findings are important when considering population-specific molecular profiles for the development of precision medicine strategies for assessing PCa risk. They align with the principles of precision medicine, which emphasize creating approaches

that consider an individual's genetic background, lifestyle, environment, and family health history [64]. Future research could enhance this approach, which has the potential to guide patients in making informed health decisions and reducing healthcare costs by delivering the most effective treatments from the outset.

Thus, given the unique genetic and environmental factors affecting the Puerto Rican population, these biomarkers may enhance personalized treatment strategies aimed to control tumor growth, preventing metastasis, and managing symptoms more effectively. Specifically, N-cadherin expression was identified as a critical determinant of PCa aggressiveness, exhibiting a moderate discriminatory power. This emphasizes N-cadherin's potential as a classifier for aggressive disease, particularly in Puerto Rican PCa patients. While these findings are encouraging, a prospective study with a larger sample size from Puerto Rican patients is necessary to validate their clinical utility in PCa risk assessment. Additionally, future research should focus on evaluating N-cadherin expression levels alongside other potential biomarkers and comparing these findings across different populations.

While clinicopathological data can provide some insight into a patient's risk assessment, it lacks precision in predicting individual patient outcomes, making this study significant [57]. Relying solely on clinicopathological data can lead to failure to choose the most effective treatment and potentially harm the patient [57]. Existing PCa prognostic tests, primarily based on genomics, have limitations. They can be expensive, lack clear cost-effectiveness, and often miss the impact of post-translational modifications on gene function [58,59]. Biomarkers have the potential to overcome current prognostic test limitations by aiding tumor stratification while improving cost-effectiveness and patient prognosis. Although the IHC technique used in this study provides a visual assessment of protein expression, it indicates the presence, absence, or relative abundance of proteins. This technique also provides information about the intracellular location of protein, which is crucial for understanding tumor progression [60]. In addition, IHC is generally less expensive, and results can be obtained faster than genomic testing, which requires more complex procedures [61]. Currently, the ProMark test is the only commercially available prognostic test for PCa that relies on protein expression analysis [61]. ProMark is a biopsy-based assay that quantifies eight proteins (CUL2, DERL1, FUS, HSPA9, PDSS2, pS6, SMAD4 and YBX1) demonstrated to be relevant to PCa aggressiveness in men with Gleason Score 3+3 and 3+4 [62,63]. However, the effectiveness of ProMark in predicting PCa aggressiveness in the Puerto Rican population remains uncertain, and further research is needed to evaluate its accuracy in this specific demographic [62].

A limitation of the present study is the use of retrospective biopsy samples, which could be influenced by sample preservation methods, potentially affecting protein stability and IHC staining results. Additionally, obtaining biopsies from the same patient at different time points during disease progression is a significant challenge and may not accurately reflect dynamic changes in biomarker expression over time. Therefore, incorporating a prospective study would minimize the limitations of analyzing heterogeneous samples and facilitate the inclusion of additional patient data, such as MRI results, thus contributing to a more comprehensive understanding of aggressive PCa. Such an approach will enhance the early detection and development of management strategies specifically tailored to the Puerto Rican population. In addition, working with a limited sample size (N=23) was a limitation of our study. While we acknowledge that the small sample size limits the statistical power and complexity of multivariate or machine learning analyses, our goal was to conduct a preliminary study to identify potential biomarkers in a Puerto Rican cohort. These findings serve as a foundation for future prospective studies involving larger cohorts. Ultimately, this study provides valuable insights into the biological behavior of PCa in Puerto Rican men and demonstrates the feasibility of biomarker-based

analysis in this population. Thus, we emphasize the need for future studies with a larger Puerto Rican cohort to confirm the observed associations and improve model robustness.

5. Conclusions

In conclusion, Phospho-Rb S249, N-cadherin, β-catenin, and E-cadherin are clinically relevant as IHC-based biomarkers for assessing PCa aggressiveness in Puerto Rican men. Specifically, N-cadherin was shown to be a key classifier of tumor aggressiveness, while β-catenin and E-cadherin were associated with tumor size and grade group. Additionally, Phospho-Rb S249 expression was positively correlated with tumor size and the number of positive biopsy cores. Together, the combined expression of these biomarkers may provide a more specific stratification of PCa cases, potentially informing prognosis and therapeutic decision making. As an accessible and cost-effective alternative to genomic testing, IHC biomarkers could support the early identification of high-risk patients and guide personalized treatment strategies, particularly in resource-limited settings. Importantly, this study emphasizes the need for population-specific approaches for PCa management. Puerto Rican men may exhibit unique disease characteristics, and understanding these disparities is crucial for improving healthcare equity and prognostic accuracy across diverse populations. Future large-scale prospective studies are essential to validate these findings and to establish the clinical utility of these biomarkers in routine diagnostic workflows for PCa management.

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Data Availability Statement: The data supporting the findings of this study are available on GitHub at https://github.com/svalle19/Puerto-Rico-PCa-data.git.

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Original Article

Burnout, Well-being, and Distress Among Orthopaedic Surgeons in Puerto Rico

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Abstract: Physician burnout is a global concern, yet its prevalence among Puerto Rico's surgeons remains underexplored. This study assesses burnout among orthopaedic surgeons working within the island's strained healthcare system, marked by economic disparities, privatization, and physician exodus. A cross-sectional survey was conducted among 67 orthopaedic surgeons (over 60% of the island's workforce) and 27 romantic partners. The survey included the Maslach Burnout Inventory-Human Services Survey, General Health Questionnaire 12, and the Revised Dyadic Adjustment Scale. Burnout was identified in 28.4% of surgeons, with 58.2% reporting high emotional exhaustion and 37.3% high depersonalization. No respondents exhibited low personal accomplishment. The most frequently cited stressor was Puerto Rico's healthcare system, particularly health insurance restrictions (66.7%). Additionally, 25.4% of surgeons showed signs of mental health concerns, and 22.0% experienced relationship distress. While emotional exhaustion and depersonalization present as alarmingly high, resilience in personal accomplishment was notable. Interventions are needed to address systemic stressors while leveraging cultural factors that protect against burnout.

Keywords: Burnout; Orthopaedic Surgeons; Emotional Exhaustion; Puerto Rico; Healthcare System; Resilience

1. Introduction

Physician burnout is a growing concern that has captured the attention of the global medical community [1]. Characterized by emotional exhaustion, depersonalization, and a decreased sense of personal accomplishment, burnout not only compromises physician well-being, but can also affect patient care, potentially leading to poor outcomes and difficulties in patient-physician interactions [2,3]. Burnout rates are high among orthopaedic surgeons (32-36%) and other types of physicians (40-60%) in the United States [4,5]. Although physician burnout is recognized as a global issue, its prevalence and impacts remain unexplored in the unique context of Puerto Rico's collapsing healthcare system.

The island's healthcare sector experiences substantial economic and infrastructural challenges. The privatized healthcare system, marked by disparities with the continental United States, leaves medical providers navigating reimbursement rates from private insurance companies up to 70% lower than those on the mainland [6]. Additionally, the density of orthopaedic surgeons in Puerto Rico is notably low at 3.30 per 100,000, compared to 9.25 in the United States [7]. To further complicate the issue, the orthopaedic field faces an aging workforce, with a significant portion of the approximately 110 orthopaedic surgeons who currently practice medicine nearing retirement [8].

These dynamics are exponentiated by the aftermath of natural disasters and a low retention rate that witnesses six out of ten medical graduates opting to

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establish practice outside of the island [9]. Conditions in Puerto Rico not only fuel a physician exodus but also magnify the effects of an ongoing public health crisis, economic uncertainties, and the burden of managing a massive public debt [10]. The local government has employed efforts to counteract this marked migration, such as reducing physicians' income tax to 4.0% [11]. However, despite these interventions, the Puerto Rican College of Physicians has voiced a pressing need for further action, urging the government to declare a state of emergency in response to the ongoing physician exodus and the imminent collapse of the local healthcare system [10].

Due to the current situation in Puerto Rico, the prevailing assumption is that the stressors associated with practicing orthopaedic surgery within the conditions of the island's healthcare system could translate to a high rate of burnout among this cohort. Despite initiatives to alleviate systemic strains, the unique circumstances of Puerto Rico suggest the potential for an elevated burnout rate that has yet to be documented in scientific literature. This study aimed to address this significant gap by exploring the prevalence and dimensions of burnout among the island's orthopaedic surgeons. In this context, higher levels of burnout could have far-reaching consequences on the quality of patient care and the surgeons' well-being. By understanding burnout and some of its effects, scholars and public health officials will be better positioned to contribute to the implementation of local interventions to address the problem and care for a vital portion of the workforce in Puerto Rico's healthcare system.

2. Methodology

2.1 Study Design: This cross-sectional study utilized REDCap software to administer an anonymous online survey to orthopaedic surgeons and trainees across Puerto Rico, capturing quantitative data to assess burnout prevalence and its contributory factors. To ensure ethical compliance, informed consent was obtained from each respondent. The survey included three validated scales for quantitative data collection: the Maslach Burnout Inventory-Human Services Survey (MBI-HSS) for burnout assessment, the General Health Questionnaire-12 (GHQ-12) for mental health screening, and the Revised Dyadic Adjustment Scale (RDAS) for relationship satisfaction and distress. In addition to previously validated scales, the survey asked respondents to identify their single most stressful factor associated with practicing in Puerto Rico, as well as demographic and professional practice information providing context on respondent's backgrounds.

2.2 Participants: The study's target participants were orthopaedic surgeons currently practicing or training in Puerto Rico, identified through the island's sole residency program and using the Society of Puerto Rican Orthopaedists and Traumatologists (SPOT) directory as a master list. Participating surgeons and trainees were also asked to invite their romantic partners. Out of the 116 listed surgeons in the directory, six were excluded for practicing outside of Puerto Rico, resulting in a pool of 110 eligible practicing surgeons and 20 residents. The sample was recruited by availability using a snowball technique based on electronic correspondence, word-of-mouth, and direct office contact. To ensure comprehensive and confidential data collection, REDCap software assigned unique identifiers to all participants, safeguarding their anonymity, and enabling a thorough audit of the study's database.

2.3 Study Variables and Statistical Analysis: The three validated scales measured key variables. The MBI-HSS evaluated burnout through a 0-6 Likert scale of 22 items across three subscales: Emotional Exhaustion (EE), which assesses feelings of being emotionally overextended and exhausted by one's work; Depersonalization (DP), measuring an unfeeling and impersonal response towards recipients of one's care; and Personal Accomplishment (PA), evaluating feelings of competence and successful achievement in one's work with people [12]. The subscales are scored as High, Moderate, or Low-risk, with EE considered High-risk at scores above 27, DP at scores above 13, and PA at scores below 39. According to the instrument's criteria, burnout is identified when respondents report High-risk EE and High-risk DP or PA. Alternatively, the GHQ-12 assessed general mental health via a 0-3 Likert scale of 12 items. Each

item was scored based on its direction, either positively or negatively, where a total score greater than 13 indicated psychological impairment [13, 14, 15]. Finally, the RDAS measured relationship distress through a 0-5 Likert scale of 14 items across three subscales: Consensus, which assesses the level of agreement on important matters; Satisfaction, gauging the overall contentment within the relationship; and Cohesion, evaluating the sense of togetherness and emotional closeness shared by partners. Scores range from 0 to 69, with higher scores denoting greater relationship satisfaction. A cut-off of 48 distinguished non-distressed from distressed relationships, providing a quantitative measure on relationship health [16]. Efforts to minimize bias included the use of validated scales, maintaining participant anonymity, ensuring diverse recruitment, and excluding incomplete survey entries. Statistical analyses using GraphPad Prism included descriptive statistics, T-tests to compare RDAS results between surgeons and their partners and prevalence rates were calculated as percentages.

3. Results

3.1 Participant Characteristics: The study received 94 complete responses, including 67 from orthopaedic surgeons and 27 from their romantic partners. Table 1 summarizes the demographic and professional characteristics of surgeons and trainees. Participants represented a range of subspecialties, most commonly Reconstruction, Sports Medicine, and Pediatrics. The majority practiced in private settings although a small subset indicated practicing in more than one setting. Relationship status varied, with most respondents reporting being in committed relationships. Table 1 also indicates how many participants in each subgroup met the MBI-HSS criteria for burnout, which is further discussed in the subsequent sections.

Table 1: Distribution of Burnout Among Orthopaedic Surgeons by Subspecialty, Clinical Setting, and Relationship Status

	Total Participants (n = 67)	Meet MBI-HSS Criteria (n = 19)
Subspecialty or Role		
Lower Extremity	4 (6.0%)	2
Pediatric	7 (10.4%)	0
Reconstruction	18 (26.9%)	6
Spine	5 (7.5%)	2
Sports	14 (20.9%)	0
Trauma	2 (3.0%)	2
Upper Extremity	5 (7.5%)	3
Resident	4 (6.0%)	2
Other	8 (11.9%)	2
Clinical Setting		
Academic Medicine	5 (7.5%)	1
Hospital Affiliated	4 (6.0%)	2
Private Practice	41 (61.2%)	9
Resident	4 (6.0%)	2
Multiple Settings	13 (19.4%)	5
Relationship Status		
Divorced or Widowed	8 (11.9%)	3
Married or in a domestic partnership	50 (74.6%)	13
Single (never married)	9 (13.4%)	3

3.2 Burnout Prevalence: According to the MBI-HSS scoring criteria, burnout is identified when there is High-risk EE accompanied with either High-risk DP or High-risk PA. The results revealed a burnout prevalence of 28.4% among the participating orthopaedic surgeons. The data also showed a concerning scenario when dealing with emotions. A detailed analysis of the cohort's MBI-HSS subscales (Table 2) indicated that 58.2% of respondents experienced High-risk EE, and 37.3% encountered High-risk DP. In contrast, no surgeons (0%) reported High-risk PA. In a detailed demographic for EE, 77.8% of those who were single or had never married, 62.5% of those who had divorced or were widowed, and 54.0% of surgeons married or in a domestic partnership, were categorized as High-risk EE. Furthermore, 100% of surgeons specializing in lower extremity, spine, and trauma, reported High-risk EE. For DP, our analysis revealed that 55.6% of those single or never married, 37.5% of those divorced and widowed, and 34.0% of those married or in a domestic relationship scored within the High-risk DP category. Notably, 100% of trauma, 80% of upper extremity, and 42.9% of sports medicine specialists were also classified as High-risk DP.

Table 2: Maslach Burnout Inventory - Human Services Survey (MBI-HSS) Risk Stratification and Results for Orthopaedic Surgeons Practicing in Puerto Rico

Subscales	MBI HSS Risk Stratification Scores	MBI-HSS Results by Respondents (%)	MBI HSS Subscale Statistics
EE	High: ≥27	39 (58.2%)	Median = 30
	Moderate: 17–26	20 (29.9%)	IQR: 21-38
	Low: 0–16	8 (11.9%)	(Mean = 30.3)
DP	High: ≥13	25 (37.3 %)	Median = 11
	Moderate: 7–12	31 (46.3%)	IQR: 8-15
	Low: 0–6	11 (16.4%)	(Mean = 11.6)
PA	High: 0–31	0 (0.0%)	Median = 49
	Moderate: 32–38	4 (6.0%)	IQR: 43-52
	Low: ≥39	63 (94.0%)	(Mean = 48.2)
Note	MBI-HSS criteria for burnout: High risk EE scores + High risk DP scores or High risk EE scores + High risk PA scores		
	Burnout detected: 28.4% (19/67)		

Table 2 Note: EE = Emotional Exhaustion, DP = Depersonalization, PA = Personal Accomplishment. IQR = Interquartile Range. MBI-HSS subscale scores are presented as overall descriptive statistics (median, IQR, and mean) for each domain and are not directly tied to the risk stratification tiers listed in the adjacent columns. IQR represents the middle 50% (25th percentile through 75th percentile) of the data and is useful for understanding score variability while minimizing the influence of outliers. Burnout was defined as high EE and high DP scores, or high EE and low PA scores.

3.3 Perceived Stressors: When asked to identify the main stressor as a practicing orthopaedic surgeon in Puerto Rico, a significant majority (67.2%) selected "Puerto Rico's healthcare system" as the primary source of stress (Figure 1). A subsequent question probed into the most stressful aspects of the local healthcare system, revealing that "Health insurance restrictions" were considered the most stressful component by 66.7% of those that had already identified the healthcare system as the primary stressor. Notably, the restrictions were identified over other factors such as "Lack of resources" (20.0%), "Lack of supporting medical personnel" (11.1%), and "Infrastructural limitations" (2.2%). When exploring the main stressor for surgeons with identified burnout, "Puerto Rico's healthcare system" persisted as the main stressor, followed by "Volume of patients," and "Private practice debt."

Figure 1: Distribution of Primary Stressors as Perceived by Orthopaedic Surgeons in Puerto Rico

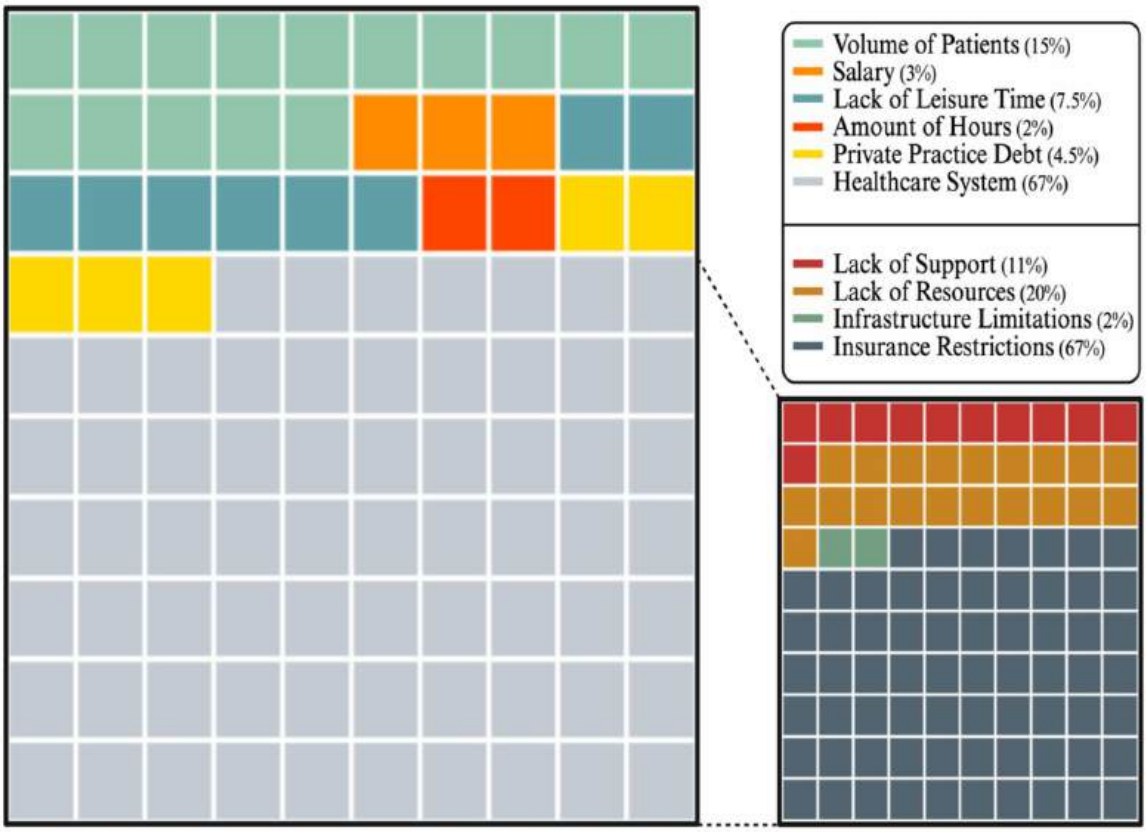

Figure 1 portrays the nested relationship between the two multiple choice questions provided to survey participants. The 10 × 10 waffle on the left shows the primary stressor selected by the entire surgeon cohort (n = 67); each square represents ~1 % of respondents. Light-grey squares (67 %) correspond to surgeons who identified "Puerto Rico's healthcare system." These squares are expanded in the 10 × 10 inset waffle on the right, where each square again equals ~1 %, but within the healthcare system subgroup, to display the specific healthcare-system issues identified (insurance restrictions, lack of resources, lack of support personnel, infrastructural limitations). Percent values for every category appear in the legend adjacent to the chart.

3.4 Mental Health and Relationship Satisfaction: Approximately 25.4% of surgeons reported symptoms suggesting the need for enhanced wellness resources, as indicated by the GHQ- 12. Of note, the results presented higher means for "Lost much sleep over worry" (1.27 ± 0.93) and "Felt constantly under strain" (1.45 ± 0.80), and lower means for "Been thinking of yourself as a worthless person" (0.22 ± 0.55) (Table 3). Furthermore, the RDAS results indicated that 22.0% of surgeons and 14.8% of romantic partners reported relationship distress (Table 4). Unpaired T tests with Welch's correction compared both surgeons and their partners for RDAS results but found no significant difference between their results.

246 **Table 3**: General Health Questionnaire-12 (GHQ-12) Response Analysis of Orthopaedic
247 Surgeons Practicing in Puerto Rico

GHQ-12 Items	Mean	SD	Response Frequencies (%)			
			0	1	2	3
Able to concentrate	0.96	0.61	0.21	0.63	0.16	0
Lost much sleep	1.27	0.93	0.21	0.43	0.24	0.12
Playing a useful part	0.64	0.62	0.43	0.49	0.07	0
Capable of making decisions	0.66	0.59	0.4	0.54	0.06	0
Under strain	1.45	0.8	0.09	0.48	0.33	0.1
Could not overcome difficulties	0.78	0.78	0.42	0.4	0.16	0.01
Enjoy day-to-day activities	1	0.82	0.27	0.52	0.15	0.06
Face up to problems	0.94	0.72	0.24	0.63	0.09	0.04
Feeling unhappy or depressed	0.9	0.86	0.36	0.45	0.13	0.06
Losing confidence	0.7	0.84	0.51	0.31	0.15	0.03
Thinking of self as worthless	0.22	0.55	0.82	0.15	0.01	0.01
Feeling reasonable happy	0.73	0.59	0.34	0.58	0.07	0
Total	10.24	6.15	Respondents at risk: 25.4% (17/67)			

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249 **Table 4**: Comparison of Relationship Dyadic Adjustment Scores (RDAS) between
250 Orthopaedic Surgeons Practicing in Puerto Rico and their Romantic Partners

Subscales	Orthopaedic Surgeons *n*=50	Romantic Partners *n*=27	Unpaired t test with Welch's correction
Consensus	Mean = 24.94	Mean = 24.96	$P = 0.9810$
Scores: 0-30	SD = 3.27	SD = 4.36	ns
Satisfaction	Mean = 15.94	Mean = 15.56	$P = 0.5149$
Scores: 0-20	SD = 2.27	SD = 2.55	ns
Cohesion	Mean = 12.76	Mean = 12.44	$P = 0.7588$
Scores: 0-19	SD = 4.31	SD = 4.26	ns
Total RDAS	Mean = 53.64	Mean = 52.96	$P = 0.7593$
Scores: 0-69	SD = 8.52	SD = 9.49	ns
Note	**Distress detected: 22.0% (11/50)**	**Distress detected: 14.8% (4/27)**	RDAS Criteria for Distress: ≥48 is considered Non-distress ≤47 is considered Relationship Distress

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4. Discussion

The rationale for this study was based on the expectation that, due to systemic difficulties of Puerto Rico's local healthcare system, we would uncover an elevated prevalence of burnout among this cohort [6,9,10]. Our findings reveal a complex interplay between resilience and the challenging reality of the healthcare system. In conjunction to reporting a 28.4% prevalence of burnout and operating under systemic constraints, an alarming number of orthopaedic surgeons in Puerto Rico exhibited high levels of exhaustion and depersonalization.

Within the surveyed cohort, a substantial portion of orthopaedic surgeons reported high levels of emotional exhaustion and depersonalization. Specifically, 58.2% reported high risk for emotional exhaustion, while 37.3% reported high risk for depersonalization. Despite these concerning numbers, the overall prevalence of burnout was lower than what our team anticipated. The levels of emotional exhaustion and depersonalization appear to be mitigated by a powerful sense of personal accomplishment, which could play a crucial role in buffering the potential negative effects of other burnout dimensions. It is worth mentioning that high levels of personal accomplishment among orthopaedic surgeons have been reported in other cohorts [17,18]. In our study, no surgeons reported High-risk PA levels (Table 2). This could suggest that the island's orthopaedic surgeons possess, or benefit from, unexplored factors of resilience which allow them to maintain high levels of personal accomplishment amidst challenging conditions. Additionally, these protective factors could be linked to cultural or regional attributes, such as strong community ties or a deep sense of purpose derived from serving within a resource-constrained healthcare environment, such as those reported among other Hispanic communities [19,20]. These intrinsic rewards may also offset the stressors typically associated with the demanding nature of orthopaedic practice [21].

The alarming rates of emotional exhaustion and depersonalization might be traced back to the challenges related to the practice of medicine in Puerto Rico. Respondents highlighted the local healthcare system, particularly health insurance restrictions, as a key stressor. Although this contradicts our team's initial assumption that proposed a high volume of patients as a potential factor, given the island's low surgeon-to-population ratio, it nonetheless draws attention to the impact of systemic constraints on the well-being of its orthopaedic workforce [7]. The focus now shifts from mere numerical scarcity to a more complex reality of healthcare delivery in Puerto Rico, creating avenues for understanding and addressing these perceived stressors.

Although the results from the GHQ-12 revealed that approximately one in every four surgeons reported symptoms indicative of mental health concerns, it does not appear to significantly impair their perceived professional efficacy. Furthermore, the notably low incidence of relationship distress among surgeons (22.0%) and the surveyed partners (14.8%) could suggest that, while burnout and stress can affect aspects of personal life, the surgeons and their families may be employing successful coping strategies or benefiting from robust support systems. These findings, particularly in the context of Hispanic/Latino culture known for close familial ties and strong community support, underscore the potential of cultural resilience in mitigating the impacts of professional stress [19,22]. This aspect warrants further exploration, as it may offer insights into protective factors that buffer against the adverse effects of burnout, both in personal and professional settings.

As a survey-based study containing both validated and non-validated tools, our study's limitations should be considered when interpreting the results. A notable constraint was our decision to prioritize participant confidentiality over the collection of specifiers pertaining to age or sex, which constrained our ability to perform a more detailed demographic analysis. The focus of our research was orthopaedic surgeons currently practicing in Puerto Rico, intentionally excluding those practicing in the continental United States or abroad, which could influence the generalizability of our findings. Additionally, the cross-sectional nature also

restricts the ability to explore burnout over time or to establish causal relationships. Moreover, the reliance on self-reported measures introduces the potential for bias, and the possibility of non-response among eligible orthopaedic surgeons and their partners may affect the representativeness of the results. However, despite these limitations, our data is consistent with that of other studies that relied on similar methodology and tools, suggesting the validity of our methods [23,24,25].

5. Conclusions

Our study unveils critical insights into the well-being of Puerto Rico's orthopaedic surgeons, revealing alarmingly high rates of emotional exhaustion and depersonalization despite a prevalence of burnout that is slightly lower than those reported among other cohorts [20]. These findings point to the profound impact of systemic constraints, such as health insurance restrictions and the privatized healthcare system, on the mental health of these healthcare professionals. Simultaneously, the findings highlight the crucial role of cultural and regional attributes in fostering the resilience exhibited. Although personal accomplishment remains high, the incidence of exhaustion and depersonalization emphasize the urgent need for interventions targeted at alleviating the toll of these systemic constraints. This study lays the groundwork for future research aimed at understanding how systemic stressors uniquely affect Puerto Rico's healthcare professionals. Ultimately, this study not only adds to the existing body of knowledge on physician burnout but also invites a more nuanced approach to developing support systems that are culturally sensitive and contextually relevant.

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REVIEW ARTICLES

Review Article

Hermansky-Pudlak Syndrome: A Bilingual Assessment of Quality and Readability of Online Health Information

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Abstract: Hermansky-Pudlak Syndrome (HPS), characterized by oculocutaneous albinism and a bleeding diathesis, is an autosomal recessive disorder particularly found in Puerto Ricans. The highest prevalence is noted in the northwest of Puerto Rico, where one in 1,800 individuals are affected. Due to its complex presentation and treatment regimen, health information must ideally be accessible, understandable, and of high quality. We selected three keyword phrases in English and three in Spanish that describe the disease in layman's terms and entered them as prompts in a *Google* search to simulate patient-initiated searches. The first 20 websites for each of the six terms were analyzed for quality and readability utilizing a DISCERN instrument questionnaire and an online readability test tool, respectively. The results for the English terms yielded a mean general reliability of 72.8%, with the quality and reliability of treatment information at a mean of 37.4%. The average grade level (AGL) recommended for the English sites was 14, with academic and private/commercial sites scoring 15 and 13, respectively. For the Spanish keywords, the general mean reliability was 65.8%, and the treatment information mean score was 27.4%. The recommended AGL for Spanish websites was 16. Although information on HPS is readily available, our analysis suggests that the complexity of the content may be high, with extreme variations in quality. This phenomenon was observed in both languages. Our results highlight the need to provide understandable, simple, and reliable quality information in both languages for educational purposes.

Keywords: Hermansky-Pudlak Syndrome; Online Health Information; Albinism; Quality and Readability; Hispanic; Genodermatosis; Bilingual.

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1. Introduction

Hermansky-Pudlak Syndrome (HPS) is an autosomal recessive disorder affecting up to nine per 1,000,000 individuals. HPS is characterized by oculocutaneous albinism and a bleeding diathesis [1]. Currently, there are 11 known genes responsible for HPS: *P3B1*, *AP3D1*, *BLOC1S3*, *BLOC1S5*, *BLOC1S6*, *DTNBP1*, *HPS1*, *HPS3*, *HPS4*, *HPS5*, *HPS6*. The mutated genes affect melanosome biogenesis, platelet-dense bodies, and lysosomes [1,2]. Each subtype of HPS can present with a diverse set of symptoms and different magnitudes of severity. For example, individuals with mutations in *HPS1* are prone to spontaneous bleeding and marked albinism. Meanwhile, individuals who carry *HPS3* mutations exhibit mild skin hypopigmentation, and although spontaneous bleeding is not as common as in *HPS1* patients, it can occur. In addition, they are equally predisposed to bleeding complications during surgical procedures [3]. Other important symptoms include nystagmus, pulmonary fibrosis, and granulomatous colitis [1]. In addition, HPS patients suffer from significant vision impairments that hinder their ability to perform daily activities. HPS is most prevalent among Puerto Rican populations due to a founder's effect, a 16-bp duplication in exon 15 of the HPS gene that explains the phenomenon observed in Puerto Rican patients with the disease. However, the absence of this mutation does not exclude an HPS diagnosis, as the syndrome likely displays locus heterogeneity [3,4]. Because of the founder's effect, the prevalence of HPS is highest in the northwest of Puerto Rico, where approximately one in 1,800 persons are diagnosed with HPS, and approximately one in 22 is a carrier of the gene [4].

In the context of HPS treatment in Puerto Ricans, the available options can be limited. These patients often face socioeconomic challenges that can impede the management of bleeding disorders, skin cancer, and gastrointestinal and pulmonary complications. For example, *HPS1* patients typically have a fatal prognosis once they are diagnosed with pulmonary fibrosis; the only definitive cure is a lung transplant, which is unavailable in Puerto Rico. Many patients in rural areas of Puerto Rico face significant challenges in accessing medical care, as they often must drive for hours to reach specialized providers in metropolitan regions. This is further complicated by the fact that many HPS patients are legally blind, making it impossible for them to drive themselves to appointments. As a result, they are often dependent on others for transportation, compounding the difficulty to meet their healthcare needs.

Notably, the decision-making process for individuals with HPS involves careful consideration of available treatment options, consulting with family members, and a personalized assessment of risks and benefits. This highlights the fact that the availability of quality information from physicians and online resources regarding HPS is crucial for patients considering such interventions. HPS patients and their families have varying educational levels and resources, which is why this creates a particular need to create accessible and understandable online information that can benefit all. In this paper, we evaluated the quality and readability of both Spanish and English online health information (OHI) regarding HPS using validated instruments.

2. Materials and Methods

We used a previously existing methodology for OHI analysis to assess the quality and readability of HPS's OHI [5,6]. First, we selected three key terms in English and three key terms in Spanish that represented the disease in layman's terms. Then, each of these terms was utilized as a prompt in a *Google* search—simulating the online search of an average HPS patient seeking medical information about their disease after a formal diagnosis. The Spanish terms were: 'Síndrome de Hermansky-Pudlak,' 'Albinismo y sangrado,' and 'Albino en Puerto Rico.' The corresponding English terms were: 'Hermansky-Pudlak syndrome,' 'Albinism and bleeding,' and 'HPS Puerto Rico.'

Quality: The first 20 websites that appeared for each of the six terms were analyzed for quality using an augmented DISCERN analysis [7]. This tool assessed website reliability, treatment options, and disease background. Repeated websites were only counted once. Two authors were assigned to the English terms and Spanish terms, respectively. A 5-point scale was used to rate website reliability and treatment option metrics. The average was calculated and converted into a percentage for each set of metrics. Disease background was evaluated dichotomously with 'yes' or 'no' scales for content. A third author intervened if discrepancies between the two authors were present in these scales. The DISCERN scores were broken down and categorized depending on the type of website, such as academic, advocacy/nonprofit, government, and private/commercial. ANOVA testing compared website reliability and treatment information across different site types for websites in English or Spanish, respectively, with $\alpha = 0.05$. Subsequent post-hoc analysis using Tukey's Honestly Significant Difference (HSD) Test was performed for significant results.

Readability: The first 20 websites that appeared for each of the six terms were analyzed for readability with a readability test tool [8]. The following formulas were used to evaluate each website: Flesch-Kincaid Grade Level (FKGL), the SMOG index, and the Coleman-Liau index (CLI). These formulas are based on assessing the number of syllables, characters, text cohesion, or choice of words. When combined, these formulas can generate a grade-level index. These scores can provide a reference to the understandability and complexity of a text and the approximate years of education needed to understand it.

3. Results

The DISCERN analysis for the English terms yielded reliability scores ranging from 32.5% to 97.5%, with a mean reliability of 72.8%. The quality and reliability of the treatment information provided by these terms yielded scores ranging from 24.3% to 67.1% and a mean of 37.4%. In terms of readability scores for the English terms, the AGL recommended to fully understand the content of the sites was 13. AGL recommendations based on site type resulted in academic sites scoring 15, while private/commercial sites scored 13. Interestingly, no government or advocacy/non-profit sites were found when the search was conducted using these terms.

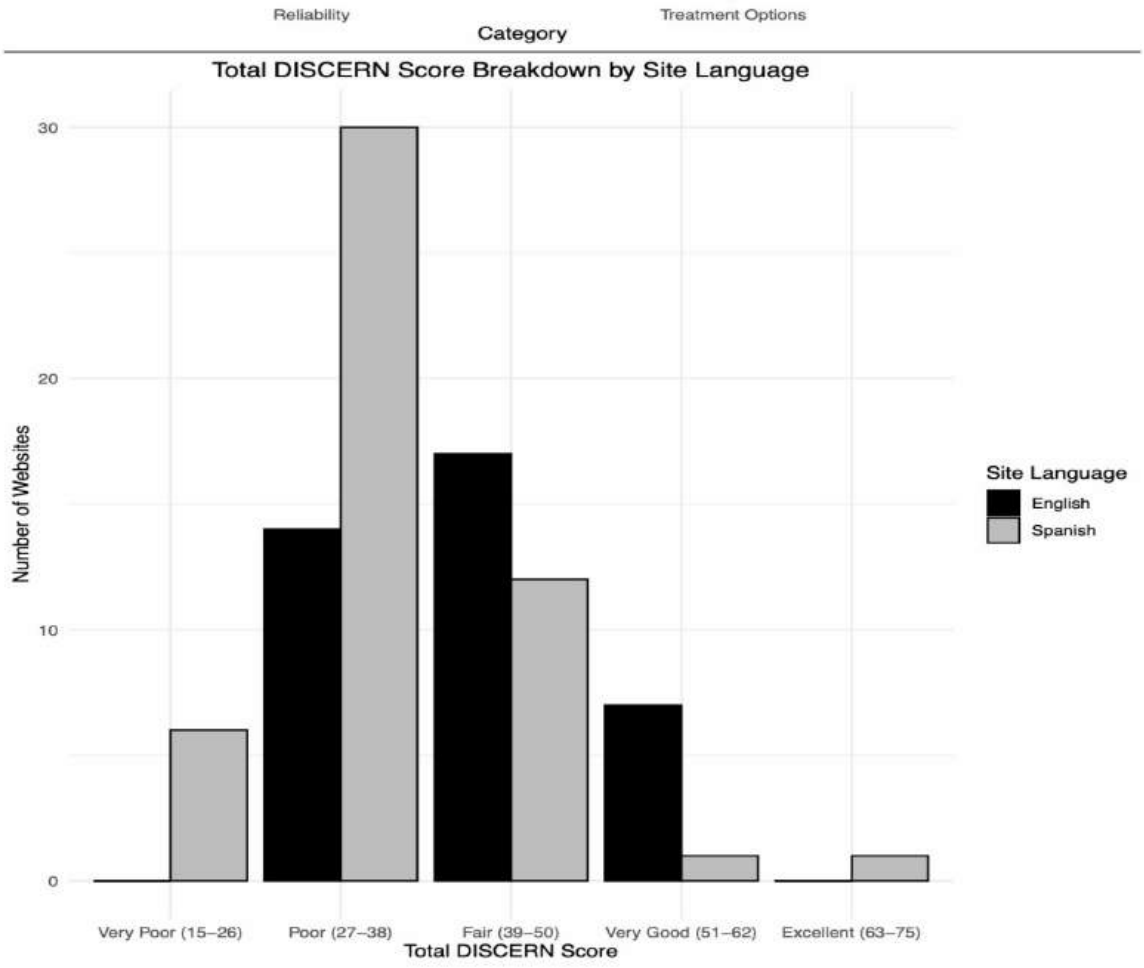
When considering the Spanish keywords, DISCERN reliability scores ranged between 38.8% to 98.8%, yielding a mean reliability of 65.8%.

Quality and reliability scores for treatment information ranged between 21.4% to 72.9%, with a mean score of 27.4%. The recommended AGL for the Spanish sites was 16. In terms of AGL, an increasing trend was noted depending on the type of site. Academic sites recommended the highest grade level (20), followed by advocacy/nonprofit sites (15), private/commercial sites (14), and lastly, government sites (12.7). In terms of the quality of analyzed websites, DISCERN reliability scores ranged from 26.3% to 96.3%. DISCERN treatment information scores ranged from 27.1% to 81.4%. Mean DISCERN scores were 58.9% and 51.7% for website reliability and treatment sections, respectively.

Out of all sites analyzed, most search results were academic (42 websites), with 43.2% of the search results falling into this category. The remaining sites were 31.8% commercial/private (26 websites), 14.8% advocacy/nonprofit (13 websites), and 10.2% government (10 websites). DISCERN values for website reliability and treatment information for each site type by language, English or Spanish, were measured (**Table 1**). Commercial/private websites scored highest on DISCERN for reliability and treatment for English search terms. No significant differences in quality of reliability or treatment information across different website types were noted with English search terms. For Spanish search terms, reliability of academic websites was significantly lower than advocacy/nonprofit websites ([p = 0.0298], 95% C.I. = [0.977, 0.0719])), and government websites ([p = 0.01], 95% C.I = [0.999, 0.190]), and additionally overall reliability of government websites were significantly higher than commercial/private websites ([p = 0.0351], 95% C.I. = [-0.764, -1.488]). Likewise, for Spanish search terms, treatment information from academic websites scored significantly lower than advocacy/nonprofit websites ([p = 0.000177], 95% C.I. = [0.929, 0.393]), and advocacy/nonprofit websites scored significantly higher than commercial/private websites ([p = 0.000674], 95% C.I. = [-0.775, -1.266]).

There was a large variability in the quality of analyzed websites. DISCERN website reliability scores ranged from 26.3% to 96.3%. DISCERN treatment information scores ranged from 27.1% to 81.4%. Mean DISCERN scores were 58.9% and 51.7% for website reliability and treatment sections, respectively (**Figure 1**).

190 **Figure 1.** Total DISCERN Score Breakdown by Site Language



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194 **Table 1.** Website reliability and treatment information for each site type by language.

Website Type	English Reliability Mean	English Reliability SD*	English Treatment Mean	English Treatment SD*	Spanish Reliability Mean	Spanish Reliability SD*	Spanish Treatment Mean	Spanish Treatment SD*
Academic	3.6	0.6	1.7	0.7	2.8	0.6	1.1	0.3
Advocacy/Non profit	3.8	0.6	1.7	0.7	3.8	0.9	2.1	1.0
Commercial/Private	4.2	0.1	2.2	0.9	3.1	0.6	1.3	0.2
Government	3.7	0.2	2.0	1.4	3.8	0.6	1.6	0.3

195 ***SD:** Standard Deviation

4. Discussion

While information pertaining to HPS is abundant and readily available, our in-depth analysis revealed the extreme complexity of the content with notable variations in quality. This phenomenon was observed in both the Spanish and English resources. Our findings highlight the need for English and Spanish-speaking HPS patients to have open discussions with physicians about the online health information they read. Interestingly, the readability level for Spanish websites was found to be three grades higher than that of English websites. This observation is particularly intriguing given that Puerto Rico, a predominantly Spanish-speaking country, harbors the majority of HPS patients globally. Given this context, prioritizing Spanish language accessibility for HPS online health information is paramount. Similarly, English HPS websites were found to be limited and created exclusively by academic and commercial/private entities. This finding highlights a crucial need for addressing an accessibility barrier in English OHI pertaining to HPS. Overall, most websites in both languages were from academic institutions, requiring readers to have a higher AGL to fully understand the information provided.

Other studies reported in the literature assessing the quality and readability of online health information have highlighted the significance of addressing information gaps among patients with low health literacy [5,6,9-11]. These investigations reveal that websites providing medical information to the average patient in the United States frequently exhibit poor quality, readability, and comprehensiveness. The authors underscore the importance of addressing these shortcomings to empower patients in making informed decisions regarding their health. Access to accurate and reliable medical information is, therefore, fundamental to facilitating informed healthcare decisions and ultimately improving health outcomes.

5. Conclusions

This study is the first in the literature to assess the available OHI for HPS patients and their communities. Our analysis emphasizes the necessity of providing information that is not only easily understandable but also free from unnecessary complexity to ensure consistent and reliable resources for these patients. This is particularly crucial for educational purposes, highlighting the importance of delivering accessible, trustworthy, and high-quality information to foster a better understanding of HPS across diverse linguistic communities. As previously noted, diagnosing and managing comorbidities associated with HPS poses a challenge for physicians and patients alike [12]. HPS patients require holistic care and a multidisciplinary approach, which may not always be readily accessible due to physician shortages in the northwestern region of PR. Most physicians in PR are concentrated in metropolitan areas, resulting in a saturation of medical resources in these regions, while most patients with HPS reside in more remote locations, far removed from such healthcare accessibility. It is safe to say that OHI resources can be potentially lifesaving for the distant HPS communities, making it imperative to provide the highest quality of information possible.

Looking ahead, further research should focus on enhancing website inclusivity for individuals with HPS-related disabilities, such as blindness. This can be achieved by increasing font sizes, incorporating visual

descriptions, and ensuring user-friendly navigation for both caregivers and patients [10]. Moreover, attention should be given to developing multimedia resources specifically tailored for the HPS community, such as audio descriptions and video tutorials that explain HPS-related information in accessible formats. Implementing feedback mechanisms from users with HPS can help identify barriers and improve the overall user experience. By prioritizing these enhancements, we can create a more inclusive digital environment that effectively supports the needs of individuals with HPS.

Author Contributions:

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All authors- LOL, KSS, SM, TH, KN, GS, MP, RM, SSM, and VN- have read and approved the final manuscript.

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Review Article

Semaglutide and Tirzepatide: Revolutionary Therapies for Obesity and Type 2 Diabetes Mellitus Management

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Abstract: Obesity and Type 2 Diabetes Mellitus (T2DM) represent significant global health challenges, contributing to high morbidity and mortality due to a combination of lifestyle, dietary, and genetic factors. Traditional management strategies, including lifestyle modifications, pharmacotherapy, and bariatric surgery, often fail to achieve sustained weight loss and glycemic control or are overly invasive. This review explores the potential of semaglutide (a glucagon-like peptide-1 [GLP-1] receptor agonist) and tirzepatide (a dual GLP-1/glucose-dependent insulinotropic polypeptide [GIP] receptor agonist) as transformative therapies for obesity and T2DM management. These agents act by stimulating glucose-dependent insulin secretion, suppressing glucagon release, delaying gastric emptying, and reducing appetite, resulting in significant weight reduction and improved glycemic control. Tirzepatide further enhances these benefits through dual receptor activation, promoting fat oxidation and additional metabolic benefits. Clinical trials, such as the STEP and SURPASS programs, demonstrate that semaglutide and tirzepatide achieve weight loss of up to 20.9% of initial body weight, comparable to bariatric surgery, and HbA1c reductions exceeding 2%, surpassing the 0.5% to 2% reductions typically observed with other T2DM therapies. These agents also improve cardiometabolic health and quality of life. Both drugs are generally well-tolerated, with gastrointestinal issues being the most common side effects. These therapies redefine obesity and diabetes as treatable chronic conditions, transforming metabolic health management. This review examines their mechanisms of action, efficacy, and safety, underscoring their potential to revolutionize the approach to these conditions.

Keywords: Semaglutide, Tirzepatide, GLP-1 receptor agonist, GIP receptor agonist, Obesity, Type 2 Diabetes Mellitus

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1. Introduction

Obesity and T2DM represent significant global health challenges, contributing to high morbidity and mortality. According to the World Health Organization (WHO), obesity rates have increased fourfold since 1990, with the prevalence of prediabetes, diabetes, hypertension, metabolic syndrome, cardiovascular disease, and nonalcoholic fatty liver disease increasing correspondingly due to lifestyle and diet [1]. WHO defines obesity as excessive fat accumulation that presents a risk to health, characterized by a body mass index (BMI) greater than 30 kg/m² [1]. T2DM is a chronic metabolic disease characterized by elevated levels of blood glucose resulting from insulin resistance, which over time leads to serious

glucose resulting from insulin resistance, which over time leads to serious damage to the heart, blood vessels, eyes, kidneys, and nerves [2]. Traditional strategies for managing obesity have focused on lifestyle modifications such as various types of exercise and diets [3]. Other approaches include behavioral interventions, pharmacotherapy, and surgical procedures such as bariatric surgery. Pharmacotherapy options encompass drugs like liraglutide, lorcaserin (a 5-hydroxytryptamine 2C [5-HT2C] serotonin receptor agonist), naltrexone-bupropion, orlistat, phentermine, and phentermine-topiramate [1, 3, 4]. However, despite the effectiveness of these methods, the limited available therapeutic options have prompted researchers to explore other drugs [4]. Additionally, achieving sustained long-term weight loss and glycemic control without subsequent weight regain remains challenging for many individuals, highlighting the need for more effective treatment options [3]. This review aims to comprehensively evaluate whether glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) receptor agonists, such as semaglutide (Ozempic, Wegovy) and tirzepatide (Mounjaro, Zepbound), offer a transformative approach to treating obesity and T2DM by assessing their effects, benefits, and risks. We will examine their mechanisms of action, clinical efficacy, safety and side effects, broader applications, economic implications, and comparative effectiveness to other approaches.

2. Methodology

2.1 Search Strategy

A comprehensive literature search was conducted across PubMed, Embase, Cochrane Library, Google Scholar, Scopus, *Cureus*, StatPearls, and *Frontiers in Endocrinology* to identify reliable, peer-reviewed studies and reviews focusing on semaglutide and tirzepatide for obesity and T2DM management. The search terms included "semaglutide," "tirzepatide," "GLP-1 receptor agonist," "GIP receptor agonist," "obesity treatment," and "type 2 diabetes." No date restrictions were applied to ensure the inclusion of both foundational and the most recent studies.

2.2 Article Selection

Eligible studies included randomized controlled trials, cohort studies, meta-analysis and systematic reviews that evaluated weight loss, HbA1c reduction, safety profiles, contraindications, drug-drug interactions, broader applications, economic implications, and comparison of semaglutide and tirzepatide. Inclusion criteria required full text availability in English with a focus on research and human clinical trial data. Exclusion criteria included case reports and expert opinions. Articles were screened by the author based on titles, abstracts, and full texts.

2.3 Clinical Trials Evaluation

Key clinical trials, including the STEP (Semaglutide Treatment Effect in People with Obesity) and SURPASS programs, were analyzed to assess the efficacy of these therapies in patients with and without T2DM. Outcomes such as weight loss, glycemic control, cardiometabolic health, and potential side effects were evaluated.

3. Mechanism of Action

3.1 GLP-1 and GIP Receptor Agonists

GLP-1 receptor agonist drugs were initially developed to take advantage of GLP-1 role in glucose homeostasis. GLP-1 is a hormone produced in the gut and synthesized by L cells in response to nutrient intake within the small intestine, which stimulates insulin release from the pancreas in a glucose-dependent manner by beta cells. By activating GLP-1 receptors, drugs like semaglutide regulate hemoglobin HbA1c levels, which serve as a marker of long-term blood glucose control, by enhancing insulin secretion, suppressing glucagon release, and improving insulin sensitivity. The improvement in insulin sensitivity occurs indirectly, as a result of the weight loss caused by these receptor agonists, rather than through the direct activity of GLP-1 receptor agonism.

Additionally, these drugs delay gastric emptying and stimulate receptors involved in appetite suppression and energy expenditure. Taken together, these effects contribute to the effectiveness for weight loss and glucose levels reduction [1, 5]. These effects can be enhanced by the activation of the GIP receptors, amplifying the effects observed with GLP-1 agonists alone. GIP also stimulates insulin release but operates through distinct receptors compared to GLP-1, enhancing the scope of insulin regulation and appetite control. GIP is secreted by K cells, specialized enteroendocrine cells primarily located in the duodenum and proximal jejunum. In response to food intake, GIP binds to its receptors, which are predominantly found in pancreatic β-cells, stimulating insulin release and contributing to glucose homeostasis. Unlike GLP-1, GIP also impacts adipose tissue by promoting fat storage in a nutrient abundant state, which, when combined with GLP-1 activity, amplifies insulin release and extends the appetite-suppressing effects [6, 7]. By targeting both receptors, drugs like tirzepatide increase insulin secretion more robustly, enhance fat oxidation, and further reduce appetite. Additionally, GIP agonists similar to GLP-1, also slow gastric emptying, which prolongs digestion time and decreases glucose spikes.

3.2 A Broader Scope

Enhanced insulin secretion in a glucose-dependent manner increases insulin biosynthesis allowing glucose to be efficiently transported into cells. This prevents its conversion to fat and promotes satiety, primarily through GLP-1 activity in the hypothalamus, thereby reducing appetite. Suppressed glucagon release limits glucose output from the liver, regulating blood sugar and preventing fat mobilization. Improved insulin sensitivity enables cells to absorb glucose with less insulin, lowering the tendency to store excess glucose as fat and encouraging its use for immediate energy needs. Taken together, this leads to calories reduction and subsequent weight loss, making GLP-1 and GIP agonists beneficial in weight management [8, 9].

4. Results

4.1 Clinical Trials: Semaglutide Treatment Effect in People with Obesity (STEP)

The STEP program was conducted to assess the clinical implications of using semaglutide 2.4 mg in individuals with obesity. Through clinical trials, the STEP program has provided valuable insights into the effectiveness and safety of semaglutide. The program includes STEP 1 through STEP 8, with STEP 1 to STEP 4 included in this review. STEP 1 was a 68-weeks randomized, placebo-controlled study involving 1,961 adults with a BMI of ≥30 kg/m² (or ≥27 kg/m² with at least one weight-related comorbidity) without type 2 diabetes. Participants received either once-weekly 2.4 mg semaglutide or placebo, along with lifestyle counseling every four weeks. Individuals treated with semaglutide lost an average of 14.9% of baseline weight, compared to 2.4% in the placebo group, with 32% of those on semaglutide achieving at least 20% weight loss versus only 1.7% in the placebo group. Semaglutide also significantly improved cardiometabolic risk factors, including reductions in waist circumference, blood pressure, blood glucose, LDL cholesterol, triglycerides, and inflammation markers. Moreover, in an extended STEP 1 clinical trial examining the rebound effects of discontinuing semaglutide after one year, researchers found that participants regained about two-thirds of the weight they had lost within a year of stopping the medication [10].

In STEP 2 1,210 patients with T2DM (HbA1c 7-10% and BMI ≥27 kg/m²) were randomized to once-weekly semaglutide 2.4 mg, semaglutide 1.0 mg (approved for type 2 diabetes), or placebo for 68 weeks, including 16 weeks of dose titration. Participants also received lifestyle counseling every four weeks. In this STEP 2 trial, the semaglutide 2.4 mg group lost 9.6% of initial body weight, significantly more than the 7.0% with semaglutide 1.0 mg and 3.4% with placebo. This reduction in weight was observed in all participants who completed the trial. This group also achieved greater reductions in waist circumference, fasting glucose, blood pressure, triglycerides, inflammation, and improved physical functioning and quality of life scores. Specifically, the difference in the HbA1c change at week 68 was -0.1% between semaglutide 1 mg and 2.4 mg. Both doses lowered HbA1c by more than 1% (1.6-1.7%) compared to placebo (0.4%). This indicates improved blood glucose control, resulting in lower blood glucose levels over time. Additionally, STEP 2 participants who reached an HbA1c level below 7% were from 78.5% of all patients with semaglutide 2.4 mg in contrast to 26.5% that were given placebos.

The STEP 3 trial was a 68-week, randomized, placebo-controlled study of 611 adults with a BMI ≥30 kg/m² (or ≥27 kg/m² with at least one weight-related comorbidity) who did not have diabetes. Unlike STEP 1, STEP 3 included a more intensive lifestyle intervention, with an initial 8-week meal replacement diet and 30 lifestyle counseling sessions, to assess if this approach would enhance weight loss with semaglutide 2.4 mg. Participants on semaglutide with intensive behavioral therapy lost 16.0% of their initial weight by week 68, compared to 5.7% with placebo. The semaglutide group also had greater reductions in waist circumference, blood pressure, inflammatory markers, HbA1c, and most lipid levels (except HDL), although physical function improvements did not differ significantly between groups. This clinical trial has demonstrated that patients who follow structured dietary and exercise regimens while on

these drugs achieve greater weight loss and improvements in metabolic health compared to those on pharmacotherapy alone, offering a greater synergistic effect. The STEP 4 trial assessed the effects of continuing versus stopping semaglutide after 20 weeks of initial treatment in adults with a BMI ≥30 kg/m² (or ≥27 kg/m² with at least one weight-related comorbidity) without diabetes. Initially, 902 participants received semaglutide weekly, and after 20 weeks, 803 reached the maintenance dose of 2.4 mg/week, achieving an average weight loss of 10.6%. These participants then entered a 48-week trial, where they were randomly assigned to continue semaglutide or switch to a placebo. Those who continued semaglutide lost an additional 7.9% of their week-20 weight, while those switched to placebo regained 6.9%, resulting in an estimated treatment difference of -14.8 percentage points [11]. From the start, cumulative weight loss at week 68 was 17.4% for the semaglutide group versus 5.7% for the placebo group. Continuing semaglutide also led to greater improvements in waist circumference, blood pressure, HbA1c, glucose, insulin levels, lipid profile, and physical functioning *(Table 1)* [9, 11, 12].

Table 1: Summary of Semaglutide Clinical Trials.

Trial	Participants	Weight Loss	Glycemic Control
STEP 1	1,961 adults with BMI ≥30 kg/m² (or ≥27 kg/m² with comorbidities), no diabetes	14.9% weight loss vs. 2.4% (placebo); 32% achieved ≥20% weight loss	Improved glucose markers; reduced blood pressure, LDL, and triglycerides
STEP 2	1,210 patients with T2DM (HbA1c 7-10%) and BMI ≥27 kg/m²	9.6% weight loss (2.4 mg) vs. 7.0% (1.0 mg) vs. 3.4% (placebo)	HbA1c reduction: 1.6-1.7% vs. 0.4% (placebo)
STEP 3	611 adults with BMI ≥30 kg/m² (or ≥27 kg/m² with comorbidities), no diabetes, with intensive lifestyle intervention	16.0% weight loss vs. 5.7% (placebo)	Improved A1c, blood pressure, and lipid profile
STEP 4	902 adults with BMI ≥30 kg/m² (or ≥27 kg/m² with comorbidities), no diabetes	17.4% cumulative weight loss with continued semaglutide vs. 5.7% (placebo)	Maintained HbA1c and metabolic improvements

4.2 Clinical Trials: SURPASS

The SURPASS trials assessed the safety and efficacy of tirzepatide for managing T2DM in diverse populations, from monotherapy to insulin add-on therapy, including trials specific to the Japanese population. This review focuses on SURPASS 1 through 5 and SURMOUNT-1. SURPASS-1 was a 40-week, blinded trial with 478 adults with HbA1c levels of 7-9.5% and a BMI of ≥23 kg/m², managed through diet and exercise. Participants received tirzepatide (5, 10, or 15 mg) or placebo. Tirzepatide groups showed HbA1c reductions up to -2.07% and weight loss up to 9.5 kg. The 15 mg dose also showed improvements in waist circumference, fasting plasma glucose, LDL cholesterol, blood pressure, and liver enzymes. SURPASS-2 was a 40-week, open label trial that compared tirzepatide (5, 10, and 15 mg) with semaglutide (1 mg) in 1,879 participants with HbA1c of 7-10.5% and BMI ≥25 kg/m² on metformin. Tirzepatide showed superior

results, with HbA1c reductions up to -2.46% and weight loss up to 12.4 kg. The 15 mg dose achieved greater reductions in waist circumference, fasting plasma glucose, systolic and diastolic blood pressure, cholesterol, and triglycerides. SURPASS-3 was a 52-week, open label trial with 1,444 participants on tirzepatide or insulin degludec, where tirzepatide achieved HbA1c reductions up to -2.37% and weight loss up to 12.9 kg. The 15 mg dose improved waist circumference, fasting plasma glucose, and systolic blood pressure, along with favorable changes in total cholesterol and LDL-C, surpassing insulin, which led to weight gain and minimal metabolic improvements.

SURPASS-4 was a 104-week, open label study focused on 2,002 participants with increased cardiovascular risk, comparing tirzepatide to insulin glargine. Tirzepatide achieved HbA1c reductions up to -2.58% and weight loss up to 11.7 kg. The 15 mg dose further reduced fasting plasma and waist circumference, with consistent improvements in blood pressure, LDL-C, and triglycerides, significantly outperforming insulin glargine. SURPASS-5, a 40-week, open-label trial with 475 participants, comparing tirzepatide (5, 10, and 15 mg) with placebo in individuals on insulin glargine ± metformin. Tirzepatide achieved HbA1c reductions up to -2.59% and weight loss up to 10.9 kg. The 15 mg dose reduced waist circumference, fasting plasma glucose, systolic blood pressure, and improved cholesterol and liver enzymes, while the placebo group saw minimal changes. SURMOUNT-1 was a 72-week, placebo-controlled, blinded trial with 2,539 participants focused on individuals with BMI ≥30 kg/m² (or ≥27 kg/m² with comorbidities such as hypertension, dyslipidemia, or cardiovascular disease) who had previously attempted dietary weight loss. Tirzepatide produced significant weight reductions, with the 15 mg dose leading to an average weight loss of 20.9%, along with improvements in waist circumference, fasting plasma glucose, systolic blood pressure, and diastolic blood pressure. Additional benefits included LDL cholesterol reductions and triglycerides, along with moderate HDL cholesterol increases and decreases in liver enzymes ALT and AST, suggesting liver health benefits. Despite tirzepatide showing significant benefits in weight loss and blood glucose management, long-term data on weight regain after discontinuation remains limited due to the drug recent introduction in the market. These trials collectively indicate tirzepatide potential for significant improvements in HbA1c and weight loss offering a promising therapeutic approach for managing T2DM and obesity *(Table 2)* [12, 13].

Table 2: Summary of Tirzepatide Clinical Trials.

Trial	Participants	Weight Loss	Glycemic Control
SURPASS-1	478 adults with HbA1c 7-9.5%, BMI ≥23 kg/m²	Up to 9.5 kg weight loss	HbA1c reduction up to 2.07%
SURPASS-2	1879 adults with HbA1c 7-10.5%, BMI ≥25 kg/m² on metformin	Up to 12.4 kg weight loss (15 mg)	HbA1c reduction up to 2.46%

SURPASS-3	1444 adults with T2DM, tirzepatide vs. insulin	Up to 12.9 kg weight loss (15 mg)	HbA1c reduction up to 2.37%
SURPASS-4	2002 adults with increased cardiovascular risk	Up to 11.7 kg weight loss	HbA1c reduction up to 2.58%
SURPASS-5	475 adults on insulin glargine ± metformin	Up to 10.9 kg weight loss	HbA1c reduction up to 2.59%
SURMOUNT-1	2539 adults with BMI ≥30 kg/m² (or ≥27 kg/m² with comorbidities)	Up to 20.9% weight loss (15 mg)	Reductions in fasting glucose, blood pressure, and cholesterol

5. Drugs Profile, Side Effects, and Contraindications

5.1 Semaglutide

Semaglutide is a long-acting GLP-1 receptor agonist with a half-life of 65 to 184 hours, in contrast to the natural GLP-1 hormone produced endogenously in the body, which has a very short half-life of 2–3 minutes due to rapid enzymatic degradation by dipeptidyl peptidase-4 (DPP-4). It shares approximately 94% structural similarity with human GLP-1 and exerts its effects primarily through signaling pathways that regulate glucose metabolism. Major molecular components include two amino acid substitutions and one acylation that improve resistance to DPP-4 cleavage and its affinity for albumin. Semaglutide enhances insulin secretion in response to glucose intake (in a glucose dependent manner), reduces appetite, and slows gastric emptying, leading to significant weight reduction and improved glycemic control [9]. The prolonged half-life of semaglutide allows for extended dosing intervals, enabling weekly administration. It works by glucose-dependent insulin secretion and glucagon suppression, consistent with the actions of GLP-1 receptor agonists previously discussed [12].

5.2 Tirzepatide

Tirzepatide is a dual GLP-1 and GIP receptor agonist that extends this efficacy by providing an amplified effect on weight loss and blood glucose management. It is a 39-amino-acid synthetic peptide, and the 20-carbon fatty diacid moiety extends its half-life to 5 days, allowing for weekly injections. Animal models of obesity and diabetes have demonstrated that GIP and GLP-1 administration has synergistic effects in lowering body weight, food consumption, and fat mass through mechanisms previously discussed. Tirzepatide was developed due to semaglutide effectiveness, targeting both GLP-1 and GIP receptors to enhance weight loss, glycemic control, food consumption, and fat mass, establishing these drugs as potential game-changer treatments for obesity and T2DM management [12].

5.3 Side Effects and Contraindications

Semaglutide and tirzepatide are generally well-tolerated, with gastrointestinal issues such as decreased appetite, nausea, diarrhea, vomiting, and constipation being the most commonly reported side effects, particularly at higher doses [14, 15]. Hypoglycemia is rare, but occurs more frequently in patients concurrently using insulin or sulfonylureas. In the SUSTAIN-4 trial, severe hypoglycemia was reported in <1% of participants receiving 0.5 mg semaglutide and 1% of those receiving 1.0 mg semaglutide, compared to 1% in the insulin glargine group [16]. Although rare, pancreatitis has been reported in patients using semaglutide and tirzepatide. However, clinical trials and meta-analyses, including SUSTAIN-6 and tirzepatide studies, have not demonstrated a statistically significant increase in pancreatitis risk compared to placebo or other diabetes medications [17, 18]. Patients should be advised to seek medical attention if they experience persistent severe abdominal pain. Gallbladder-related disorders, such as cholelithiasis and cholecystitis, have been reported with semaglutide, though study results vary by administration route. For subcutaneous semaglutide, gallbladder events occurred in 1.4% of treated patients compared to 1.9% in the placebo group. In contrast, oral semaglutide was associated with a higher incidence of cholelithiasis (0.6%) compared to placebo (0.1%), while the risk of cholecystitis was similar. For tirzepatide, meta-analyses did not show a statistically significant increase in gallbladder or biliary diseases compared to placebo or other diabetes medications. However, a subgroup analysis suggested an elevated risk of gallbladder or biliary diseases with the 10 mg dose of tirzepatide compared to placebo or basal insulin. These events may be related to rapid weight loss or changes in gallbladder motility, though the underlying mechanisms remain unclear [17, 18]. Another emerging concern is gastroparesis, with recent evidence showing a significantly increased risk of diagnosis among patients using GLP-1 receptor agonists compared to those on bupropion-naltrexone [19]. Semaglutide has been associated with a slight increase in heart rate, though this has not been linked to adverse cardiac events. Acute kidney injury has been reported in rare cases with both treatments, often due to dehydration caused by GI side effects. However, clinical trials found similar rates of acute kidney injury between semaglutide and placebo, with long-term studies demonstrating renal protective effects in patients with diabetes [17]. Additionally, semaglutide has been linked to temporary worsening of diabetic retinopathy (DRP) in individuals with pre-existing DRP. This effect is likely attributable to rapid glucose reduction, as patients with the greatest HbA1c reductions exhibited the highest risk of DRP progression [15, 17]. According to the Food and Drug Administration (FDA), serious hypersensitivity reactions, including anaphylaxis, have been reported with the use of semaglutide and tirzepatide.

The FDA contraindicates the use of semaglutide and tirzepatide in patients with a history of pancreatitis, severe gastrointestinal disorders, acute kidney injury, acute gallbladder disease, or DRP due to the potential exacerbation of these conditions. These agents are also contraindicated in individuals with a personal or family history of medullary thyroid

carcinoma (MTC) or multiple endocrine neoplasia type 2 (MEN2), based on preclinical studies in mouse models showing an increased risk of thyroid C-cell tumors. However, this risk has not been observed in human clinical trials [15, 19].

6. Discussion

The advent of semaglutide and tirzepatide represents a transformative advancement in the treatment of obesity and T2DM. These receptor agonists have demonstrated remarkable efficacy in weight loss and glycemic control, addressing critical gaps in current therapeutic strategies. While traditional approaches such as lifestyle modifications, pharmacotherapy, and bariatric surgery remain crucial, clinical trials suggest that semaglutide and tirzepatide offer a more consistent and sustainable way to manage weight loss and glycemic control. The STEP and SURPASS trials highlight the superior efficacy of semaglutide and tirzepatide compared to other pharmacological interventions. Semaglutide has been shown to reduce weight by up to 17.4% (STEP 4) and lower HbA1c levels by 1.6–1.7% (STEP 2), which is clinically significant compared to other HbA1c-lowering drugs that typically achieve reductions of 0.5–2%. Tirzepatide, with its dual action, further enhances these effects, leading to weight loss of up to 20.9% (SURMOUNT-1) and HbA1c reductions of up to 2.59% (SURPASS-5), also surpassing the typical 0.5–2% reduction seen with other medications [20, 21]. However, challenges remain, particularly regarding weight rebound after discontinuation. Follow-up data from the STEP 4 trial indicate that discontinuation of semaglutide led to significant weight regain, with an average regain of 6.9% within 48 weeks. Similarly, an extension of the STEP 1 trial observed that participants regained approximately two-thirds of their lost weight one year after stopping semaglutide. While tirzepatide has demonstrated significant outcomes, long-term data on weight regain after discontinuation remain limited. Given its similar mechanisms of action to semaglutide, a comparable rebound effect may be expected if treatment is halted. These findings suggest that continued use of these therapies may be necessary to sustain weight loss and associated health benefits. Overall, semaglutide and tirzepatide represent promising therapeutic options for patients suffering from T2DM or obesity, with tirzepatide offering enhanced benefits for those requiring more robust metabolic improvements. Nevertheless, challenges such as potential side effects, weight rebound, drug-drug interactions, and high costs must be addressed to optimize their clinical use.

6.1 Drug-Drug Interactions

Semaglutide and tirzepatide interact with various medications due to their effects on gastric emptying, glucose metabolism, and hormonal regulation. Delayed gastric emptying can alter the absorption of oral drugs, which can include antibiotics, pain relievers, thyroid hormones, and antiepileptics, potentially reducing their efficacy. Dose adjustments or timing modifications may be necessary to ensure therapeutic effects. Additionally, these medications increase the risk of hypoglycemia when combined with insulin or sulfonylureas, necessitating careful dose adjustments to prevent severe blood glucose drops [16]. Interactions with DPP-4 inhibitors should also be considered, as both drug classes modulate

incretin signaling via GLP-1 receptor activation. While GLP-1 receptor agonists resist enzymatic degradation, DPP-4 inhibitors prolong endogenous GLP-1 activity by preventing its breakdown. Their concurrent use may lead to unexpected pharmacodynamic effects, with studies suggesting that GLP-1 receptor agonists may provide greater clinical efficacy than DPP-4 inhibitors [22]. Although some studies suggest that warfarin absorption is not affected, potential alterations cannot be ruled out. Therefore, frequent INR monitoring is recommended to prevent coagulation complications [23]. Caution is also advised with corticosteroids, as they can raise blood sugar levels, potentially reducing the effectiveness of GLP-1 receptor agonists in diabetes management. Furthermore, beta-blockers may mask hypoglycemia symptoms, complicating the recognition of low blood sugar, particularly in patients using insulin or sulfonylureas. Careful blood glucose monitoring is essential when these medications are used together.

6.2 Economic Implications

The economic implications of semaglutide and tirzepatide are significant and impactful. The high cost of these therapies, ranging from $800 to $1,400 per month depending on the therapy purpose in several markets, presents a barrier to therapy accessibility. Insurance coverage varies widely, with many insurers approving these medications for T2DM but not for obesity or weight management. International Foundation of Employee Benefit Plans survey data indicate that in 2024, 57% of insurers cover GLP-1 drugs for diabetes, 34% for both diabetes and weight loss, and none for weight loss alone. Furthermore, 65% of insurers have indicated no intent to cover GLP-1 treatments specifically for weight loss in the future. Additionally, the type of insurance provider and plan plays a crucial role, as some insurers have withdrawn coverage due to rising costs, leading to higher out-of-pocket expenses for patients. The high drug costs often discourage patients from using them, even though they are highly effective. The rapid adoption of these medications, particularly semaglutide, has significantly impacted the pharmaceutical industry. The original semaglutide and tirzepatide manufacturers, Novo Nordisk and Eli Lilly, have experienced substantial growth in market valuation, reaching hundreds of billions of dollars. Efforts to improve cost-effectiveness, such as developing generic formulations and exploring alternative mechanisms of action, are crucial to ensuring equitable access. While these drugs are already available and widely used, their broader implications and long-term outcomes continue to be explored.

6.3 Broader Applications Beyond Obesity and Type 2 Diabetes Mellitus

Currently, semaglutide and tirzepatide are being used for purposes beyond managing obesity and type 2 diabetes. These drugs are increasingly prescribed off-label for weight management in overweight individuals (BMI 25–30 kg/m^2) and even for cosmetic purposes by those seeking non-medical weight loss [24]. Other applications extend to aging-related conditions, addressing a broader range of metabolic health issues such as neurodegenerative diseases, cardiovascular diseases, kidney diseases, and musculoskeletal degenerative disorders [25]. Additionally, conditions like polycystic ovary syndrome (PCOS), nonalcoholic fatty

liver disease (NAFLD), and metabolic syndrome, which often coexist with overweight and obesity, may benefit from the metabolic advantages these drugs offer. However, further research is required to evaluate the long-term effects of off-label use on metabolic health and cosmetic outcomes.

7. Conclusions

Semaglutide and tirzepatide represent revolutionary therapies for the management of obesity and T2DM. These agents achieve two to three times greater weight loss compared to traditional pharmacological treatments, with results comparable to those observed with bariatric surgery. For glycemic control, tirzepatide has demonstrated HbA1c reductions exceeding 2%, particularly effective in patients with high baseline blood glucose levels. Their glucose-dependent mechanisms significantly reduce the risk of hypoglycemia, making them safer for long-term use compared to insulin and certain oral antidiabetic agents. Tirzepatide offers additional metabolic benefits over semaglutide, positioning it as a compelling option for patients requiring enhanced metabolic outcomes. However, future research is needed to optimize patient selection, explore long-term outcomes in obesity, T2DM, and off-label applications, and improve cost-effectiveness. Additionally, investigating broader therapeutic applications in conditions such as nonalcoholic fatty liver disease (NAFLD), polycystic ovary syndrome (PCOS), and neurodegenerative diseases could further expand their clinical use. Semaglutide and tirzepatide have already begun to transform the management of obesity and T2DM into treatable chronic conditions, offering effective alternatives to surgery and traditional therapies with limited efficacy. Their introduction into the market represents a paradigm shift in metabolic health management, with the potential to improve outcomes for millions of patients worldwide.

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CASE REPORTS

Case Report

Unraveling Leptin's Influence: Sleep Abnormalities in Obesity-Related Neurogenetics

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Abstract: The role of early-onset obesity-related genetic predisposition and leptin receptor variants have been previously studied. However, studies involving sleep-related disorders linked to a genetic predisposition, leading to obesity, and how leptin could play a role in sleep-related disorders have been limited. In this study, we explore a case of how leptin receptor variants could play a role in the relationship between obesity and sleep-related disorders. We present a case of a morbidly obese (BMI of 62.87 kg/m2) Puerto Rican teenage female with a past medical history of type 2 diabetes mellitus, hypothyroidism, essential primary hypertension, and obstructive sleep apnea (OSA), who was evaluated due to complications regarding sleeping difficulties, despite being on Continuous Positive Airway Pressure (CPAP) treatment. Genetic studies performed to assess the causes of obesity revealed BBS9 heterozygous gene for a sequence variant defined as c.396GC and heterozygous *LEPR* gene for a sequence variant defined as c.658GA, which has been associated with an increased predisposition to obesity. This case report emphasizes the value of genetic research in figuring out the root causes of obesity and its comorbidities, especially in cases of early-onset obesity and co-occurring disorders such as OSA. The discovery of genetic variations in *LEPR* and *BBS9* genes offers crucial information on potential mechanisms underlying the clinical phenotype of the patient.

Keywords: leptin receptor variants; early-onset diabetes; obstructive sleep apnea.

1. Introduction

Obesity is a complex, multifaceted condition with a rising global incidence that poses a risk for severe problems and comorbidities [1]. The regulation of calorie intake, hunger, and physical activity are all involved in the etiology of obesity [2]. Although the availability of healthcare, socioeconomic position, and underlying hereditary and environmental factors could be present, research suggests that obesity-related genetic variables account for between 40% and 70% (monogenic and polygenic causes) of obesity in humans, according to family and twin studies [4]. Previous studies have explored the role of leptin receptor variants and genetic predispositions in the early development of obesity [5]. However, studies involving sleep disorders related to a genetic predisposition leading to obesity and how leptin could play a role in sleep-related disorders have been limited. In this case, we report clinical findings and genetic analysis of an 18-year-old female patient with early-onset obesity and co-occurring disorders such as treatment-resistant Obstructive Sleep Apnea (OSA). Furthermore, we explore how leptin receptor variants could play a role in the relationship between obesity and sleep-related disorders.

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2. Case Presentation

Case of an 18-year-old female patient with early-onset obesity and co-occurring disorders such as treatment-resistant Obstructive Sleep Apnea (OSA). Polysomnography (PSG) was performed to assess the severity of OSA in this patient (**Figure 1**).

Figure 1: 18-year-old female with early-onset obesity, significant facial and central adiposity, large neck circumference, and pronounced submental fat, also displaying acanthosis nigricans.

Respiratory disturbances were associated with oxygen desaturation down to a nadir of 68% during sleep. The mean oxygen saturation during the study was 91%. The cumulative time under 88% oxygen saturation was 13.4 minutes (**Table 1-4**). Prior to starting CPAP therapy, the patient had an apnea/hypopnea index (AHI) of 22.7 events per hour. During titration, the AHI was 2.1 events per hour, and the Respiratory Disturbance Index (RDI) was 2.1 events per hour. The most appropriate setting of CPAP was 8cm H2O, with a sleep efficiency of 96.8%. There were no significant findings for central sleep apnea nor periodic leg movements (PLMs) during sleep (**Appendix 1**).

Table 1. Sleep Architecture

Lights Out Time: 21:02:19		**Lights On Time: 04:47:18**	
Total Recording Time (Min.): 465.0		# of REM Episodes:	
	Time (min.)	% of Total Sleep Time	Normal %
Stage N1 (Drowse)	5.5	3.6	5-10%

Stage N2 (Mod. Depth)	86.5	56.4	45-55%
Stage N3 (Deep/Slow Wave)	61.5	40.1	0-21%
REM (Dream)	0.0	0.0	17-28%
Total Sleep Time (Min.)	153.5	100%	>360 Min.
Latency to Sleep Onset	5.0	-	<30 Min.
Latency to Stage N2	1.0		<10 Min. from onset
Latency to REM Sleep	-		80-110 Min. (approx)
Wake After Sleep Onset	0.5		-
Sleep Efficiency*	94.8		>85-90%

This table outlines the distribution of sleep stages during the total recording time of 465 minutes. Time spent in each stage includes N1 (5.5 min, 3.6%), N2 (86.5 min, 56.4%), N3 (61.5 min, 40.1%), with no REM sleep recorded. The total sleep time was 153.5 minutes, with a sleep efficiency of 94.8%. Sleep onset latency was 5.0 minutes, latency to stage N2 was 1.0 minute, and wake after sleep onset was 0.5 minutes.

Table 2. Sleep Architecture, Titration Section

Lights Out Time: 23:44:49		**Lights On Time: 04:47:18**	
Total Recording Time (Min.): 302.5		# of REM Episodes:	
		CPAP Titration	
	Normal %	TIME (min.)	% Total Sleep Time
Total Recording	-	302.5	-
Stage N1 (Drowse)	5-10%	6.0	2.1
Stage N2 (Mod. Depth)	45-55%	157.5	53.8
Stage N3 (Deep/Slow Wave)	0-21%	26.0	8.9
Stage REM (Dream)	17-28%	103.0	35.2
Total Sleep Time (Min.)	>360 Min.	292.5	100%
Latency to Sleep Onset	<30 Min.	1.0	
Latency to Stage N2		1.0	-
Latency to REM Sleep	80-100 Min. (approx)	21.0	
Wake After Sleep Onset	-	9.0	
Sleep Efficiency*	>85-90%	96.7	

This table outlines the sleep architecture during a CPAP titration study. The total recording time was 302.5 minutes, with sleep divided into stages: N1 (6.0 min, 2.1%), N2 (157.5 min, 53.8%), N3 (26.0 min, 8.9%), and REM (103.0 min, 35.2%). Latencies to sleep onset, stage N2, and REM sleep were 1.0, 1.0, and 21.0 minutes, respectively. Sleep efficiency was 96.7%, with 9.0 minutes of wake time after sleep onset.

Table 3. Respiratory Summary

	By Sleep Stage		**By Position**		**TOTAL**
	NREM	REM	Supine	Non-Supine	
Sleep Time (Min.)	153.5	0.0	153.5	0.0	153.5
APNEA					
Obstructive	0	0	0	0	0
Mixed	0	0	0	0	0
Central	0	0	0	0	0
Total Apnea	0	0	0	0	0
Apnea Index	0.0	0.0	0.0	0.0	0.0
HYPOPNEA	58	0	58	0	58
Total Apneas and Hypopneas	58	0	58	0	58
AHI*	22.7	0.0	22.7	0.0	22.7
Flow Limitation Events (RERA)	0	0	0	0	0
RDI	22.7	0.0	22.7	-	22.7

This table details the number of apneas and hypopneas during sleep, broken down by sleep stage (NREM, REM) and body position (Supine, Non-Supine). No apneas were recorded, while 58 hypopneas occurred, all during NREM sleep in the supine position. The Apnea-Hypopnea Index (AHI) was 22.7, and the Respiratory Disturbance Index (RDI) was also 22.7. No flow limitation events (RERA) were noted.

Table 4. Respiratory Summary

TIME BETWEEN	**NREM**	**REM**	**TOTAL (SLEEP)**
90+%	2:14:38.0	0:00:0.0	2:14:38.0
80-89%	0:13:18.0	0:00:0.0	0:13:18.0
70-79%	0:01:39.0	0:00:0.0	0:00:0.0
60-69%	0:00:3.0	0:00:0.0	0:00:3.0
<60%	0:00:0.0	0:00:0.0	0:00:0.0
SAO2 NADIR	68%	-%	68%

This table summarizes the time spent at different oxygen saturation levels (SpO2) during non-REM (NREM) and REM sleep stages. The time is divided into intervals of SpO2: 90+%, 80-89%, 70-79%, 60-69%, and <60%. The nadir (lowest) SpO2 recorded during sleep was 68%.

Since this patient had a history of treatment-resistant OSA due to morbid obesity, genetic testing was performed to assess the causes of obesity (**Table 5**). This patient is heterozygous in the *BBS9* gene for a sequence variant defined as c.396GC, which is predicted to result in the amino acid substitution p.Gln132His.

Furthermore, this patient is heterozygous in the *LEPR* gene for a sequence variant defined as c.658GA, which is predicted to result in the amino acid substitution p.Val220Ile.

Table 5. Summary of Genetic Testing Results for Obesity-Related Gene Variants (*BBS9* and *LEPR*) and their Clinical Significance

Gene Transcript	Mode of Inheritance, Gene OMIM	DNA Variants, Predicted Effects, Zygosity	ClinVar ID	Highest Allele Frequency in a gnomAD Population	In Silico Missense Predictions	Interpretation
***BBS9*, NM_198428.2**	AR, 607968	c.396G>C, p.Gln132His, Heterozygous	841915	0.042% Latino	Damaging	UNCERTAIN
LEPR, NM_002303.5	AR, 601007	c.658G>A, p.Val220Ile, Heterozygous	917428	0.13%, African	Tolerated	UNCERTAIN

Mode of Inheritance: Autosomal Dominant=AD, Autosomal Recessive =AR, X-Linked=XL, ClinVar ID: Variant accession, GnomAD: Allele Frequency registered in a large population database (gnomad.broadinstitute.org). Value listed is the highest allele frequency reported within one of seven population categories recognized in gnomAD v.2.0 (The "Other" population is excluded). Missense Predictions: Summarized output (Damaging, Conflicting, or Tolerated) via Poly Phen-2, SIFT, Mutation Taster, and FATHMM.

Additionally, leptin levels were measured, and genetic testing was performed on the patient's parents. The father's leptin level was 34.2 ng/mL (Male: 1.8-19.9 ng/mL). Genetic analysis showed the father is heterozygous in the *BBS9* gene, heterozygous in the *LEPR* gene, and heterozygous in the *DNMT3A* gene (**Table 6**). On the other hand, the mother's leptin level was 64.6 ng/mL (Female: 8-38 ng/mL), and she was negative for genetic variants (**Figure 2**).

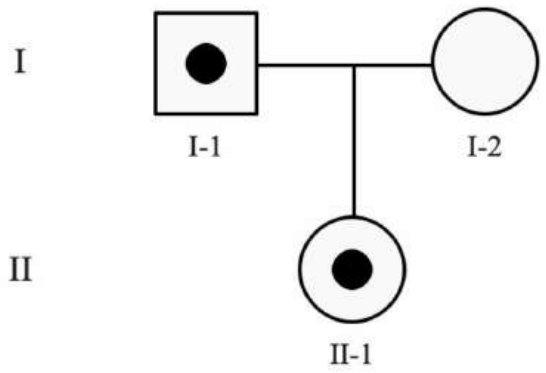
Table 6. Summary of Genetic Testing Results for Obesity-Related Gene Variants (BBS9 and LEPR) and their Clinical Significance in the Patient's Father

Gene Transcript	Mode of Inheritance, Gene OMIM	DNA Variants, Predicted Effects, Zygosity	ClinVar ID	Highest Allele Frequency in a gnomAD Population	In Silico Missense Predictions	Interpretation
***BBS9*, NM_198428.2**	AR, 607968	c.396G>C, p.Gln132His, Heterozygous	841915	0.042% Latino	Damaging	UNCERTAIN
***LEPR*, NM_002303.5**	AR, 601007	c.658G>A, p.Val220Ile, Heterozygous	917428	0.13%, African	Tolerated	UNCERTAIN
***DNMT3A*, NM_175629.2**	AD, 602769	c.835G>A, p.Asp279Asn, Heterozygous	1190119	0.0029%, Latino	Conflicting	UNCERTAIN

Mode of Inheritance: Autosomal Dominant=AD, Autosomal Recessive =AR, X-Linked=XL , ClinVar ID: Variant accession (www.ncbi.nlm.nih.gov/clinvar), GnomAD: Allele Frequency

registered in a large population database (gnomad.broadinstitute.org). Value listed is the highest allele frequency reported within one of seven population categories recognized in gnomAD v.2.0 (The "Other" population is excluded).
Missense Predictions: Summarized output (Damaging, Conflicting, or Tolerated) via Poly Phen-2, SIFT, Mutation Taster, and FATHMM [6].

Figure 2. Pedigree of Genetic Testing Results for Obesity-Related Gene Variants (*BBS9* and *LEPR*)



The pedigree shows the inheritance pattern of BBS9 and LEPR variants. I-1 (filled square) carries the identified variants, while I-2 (empty circle) has no known variants. II-1 (filled circle) represents the child with the variants, indicating a potential autosomal recessive inheritance pattern related to early-onset obesity.

3. Discussion

This case report presents the clinical and genetic findings of an 18-year-old female patient with early-onset obesity and co-occurring disorders such as treatment-resistant. What is significant about this case is how these genes could play a role in mutations of the leptin receptor gene (*LEPR*) and Bardet-Biedl syndrome 9 (*BBS9*) and how it could be related to the patient's obesity and accompanying comorbidities [7-8, 9].

The *LEPR* gene variant identified in this patient is a heterozygous sequence variant (c.658GA), resulting in the amino acid substitution p.Val220Ile. Leptin, a hormone primarily released by adipose tissue, is crucial in regulating energy balance and body weight by acting on its receptor, encoded by the *LEPR* gene [10-11]. Mutations in this variant leading to loss of function or resistance has been reported in the heterozygous state in an individual with severe obesity [12], and this variant is reported in 0.13% of alleles in individuals of African descent in GnomAD. Although we suspect that this variant may be benign, at this time, the clinical significance of this variant is uncertain due to the absence of conclusive functional and genetic evidence. Pathogenic variants in *LEPR* are associated with autosomal recessive obesity and hypogonadotropic hypogonadism due to leptin receptor deficiency [13-14]. For instance, genetic variations of the *LEPR* gene have been described in Puerto Rican children of Hispanic descent, as is the case with our patient [15].

Emerging research supports the function of leptin and its receptor in controlling energy balance and body weight, even though other genetic and environmental factors contribute to its development [5]. Leptin is primarily released by adipose tissue and circulates in the bloodstream, passing the blood-brain barrier (BBB) and acting in the brain. When leptin binds to its receptor, signaling pathways involved in energy balance and body weight control are activated [11]. Several factors (energy balance, calorie intake, adipose tissue mass, insulin levels, stress, sleep duration and quality, etc.) regulate leptin levels, particularly acute changes in energy intake [16]. In addition, rare genetic causes of severe early-onset obesity with disturbed signaling and consequent leptin resistance have been previously

reported as leptin receptor variants [7-8]. Furthermore, leptin has been previously reported to be elevated in obesity-related sleep breathing disorders such as OSA. Some research has shown that plasma leptin levels are increased in newly diagnosed, otherwise healthy individuals with untreated sleep apnea, surpassing the levels observed in similarly obese control subjects without sleep apnea [17].

Moreover, our patient carries a heterozygous sequence variant (c.396GC) in the *BBS9* gene, which has been reported in a family where individuals presented with Bardet-Biedl syndrome (BBS) [18]. BBS is a rare autosomal recessive ciliopathy characterized by retinal dystrophy, obesity, post-axial polydactyly, renal dysfunction, learning difficulties, and hypogonadism [19]. Although two additional variants in BBS1 were identified, the study did not provide segregation data to support their pathogenicity [20]. This variant is reported in 0.042% of alleles in individuals of Latino descent in gnomAD. In general, heterozygous carriers of pathogenic variants of autosomal recessive diseases present asymptomatic. The occurrence of symptomatic heterozygosity in autosomal diseases is exceptionally uncommon and has primarily been documented through individual case reports. However, certain extensive studies have indicated an elevated risk for specific diseases among individuals with one copy of the mutated gene [21]. Furthermore, BBS is characterized by notable clinical and genetic diversity. While initially conceptualized as a purely recessive trait, recent findings have revealed an oligogenic mode of disease transmission. In certain families, genetic interactions between mutations at distinct BBS loci have been identified, contributing to the causation and/or modification of the syndrome's phenotype [22]. Although heterozygous, this patient presents with a milder phenotype presentation of BBS. It has been demonstrated that a wide range of Mendelian diseases, variants traditionally considered to be recessive can cause milder phenotypes in heterozygous carriers [21].

The co-occurrence of genetic variants associated with obesity in this patient suggests a synergistic effect that can be clinically observed. Previous studies have demonstrated that certain individuals carry heterozygous mutations in multiple genes associated with interconnected biological pathways. Individually, each mutation's heterozygosity holds no clinical significance. However, when present concurrently, the heterozygosity exhibits synergy, leading to clinically relevant biochemical abnormalities. This concept of "synergistic heterozygosity" proves valuable in understanding complex phenotypes, such as seen in our patient [23].

Genetic testing was also done on the patient's parents to evaluate the inheritance pattern of the discovered variations. The *LEPR* and *BBS9* mutations found in the patient's father are indicative of an autosomal dominant or autosomal recessive inheritance pattern. The patient's mother, on the other hand, did not carry any of the detected variations yet still had an elevated leptin level, indicating that she may have developed leptin resistance without any genetic mutation. Leptin resistance is defined as the failure of leptin to promote anticipated salutary metabolic outcomes in states of over-nutrition or obesity [24]. This phenomenon has been described to be induced by multiple conditions. In general, leptin resistance can be classified based on different etiologies; like high fat diet (HFD) induced leptin resistance, inflammation induced leptin resistance, pregnancy/lactation induced leptin resistance, etc. [25]. With these findings, it can be inferred that the patient's obesity may have a paternal genetic component, as well as an environmental component.

Sleep breathing disorders associated with obesity, such as OSA, result in intermittent hypoxia during sleep, a potent inducer of oxidative stress. Elevated levels of leptin and the development of leptin resistance might enhance the production of reactive oxygen species, accelerating oxidative stress and fostering inflammation. Consequently, some studies have suggested a potential connection between leptin and oxidative stress in the development of sleep-breathing disorders [25]. Our patient presented with a treatment-resistant OSA, which could

be explained by multifactorial genetic and environmental components that have led her to chronically elevated leptin levels and obesity.

This case report emphasizes the value of genetic research in figuring out the root causes of obesity and its comorbidities, especially in cases of early-onset obesity and co-occurring disorders such as OSA. The discovery of genetic variations in the *LEPR* and *BBS9* genes offers crucial new information on putative mechanisms underlying the clinically presentable phenotype of the patient. These variations' precise clinical significance and impact on the patient's condition are still unknown. It is necessary to conduct more research, including functional investigations and extensive genetic analysis, to clarify the genotype-phenotype relationship and provide individualized treatment plans for people with obesity and related comorbidities.

4. Conclusions

Leptin dysregulation has been associated with an increased predisposition to obesity, which could lead to sleep-related disorders, such as OSA. What is novel about our case is that our patient has a *LEPR* gene heterozygous variant, leading to an increase in leptin levels, greater increase in metabolic dysregulation and increased body weight; thus, worsening her OSA. At the same time, research has shown that conditions such as OSA also increase leptin levels, which could worsen her OSA and prognosis. Further research is needed to assess *LEPR* and its role in OSA, especially in Hispanic populations, where research is limited.

Supplementary Materials: None

Author Contributions: All authors significantly contributed to the study's conception and design, clinical data acquisition, analysis, and interpretation, as well as manuscript drafting and revision, and approved the final version for publication.

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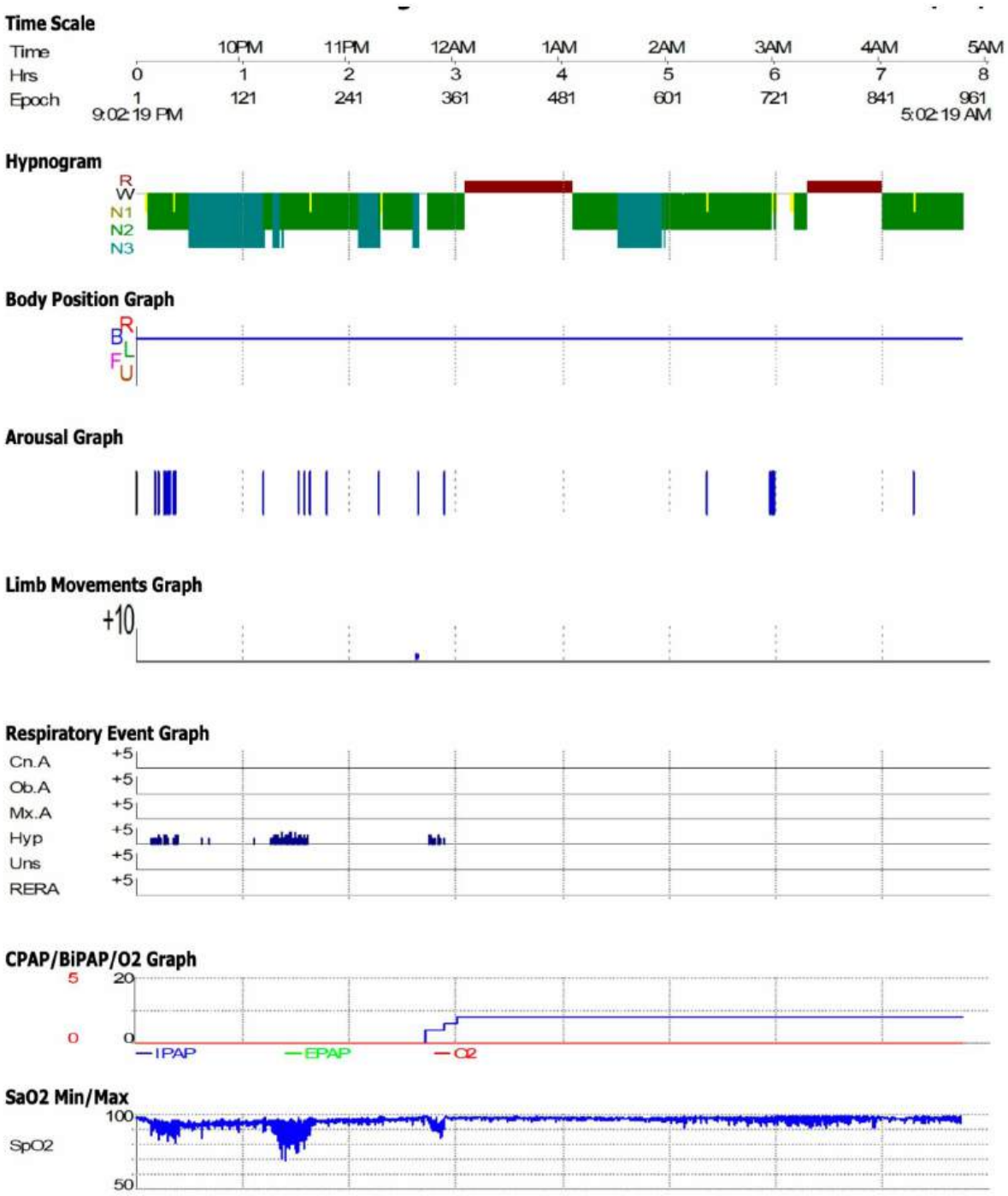
Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Ponce Health Sciences University on July 27, 2022, protocol code 2207110163.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data analyzed for this case report are included in this published article.

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix 1. Polysomnography Report with Sleep Architecture, Arousals, and Respiratory Events



Time Scale

- **Time:** Shows the hour of the night (10 PM to 5 AM).
- **Hrs:** Number of hours since the start of the recording.
- **Epoch:** Consecutive time intervals since the start of the recording.

Hypnogram

- **R:** REM sleep.
- **W:** Wake.
- **N1:** Non-REM stage 1 sleep.

- **N2:** Non-REM stage 2 sleep.
- **N3:** Non-REM stage 3 sleep.

Body Position Graph

- **B:** Back.
- **L:** Left side.
- **R:** Right side.
- **F:** Face down.
- **U:** Unknown position.

Arousal Graph

- **Vertical lines:** Indicate arousal events during sleep.

Limb Movements Graph

- **+10:** Indicates the occurrence of limb movements.

Respiratory Event Graph

- **Cn.A:** Central Apneas.
- **Ob.A:** Obstructive Apneas.
- **Mx.A:** Mixed Apneas.
- **Hyp:** Hypopneas.
- **Uns:** Unspecified events.
- **RERA:** Respiratory Effort-Related Arousals.

CPAP/BiPAP/O2 Graph

- **IPAP:** Inspiratory Positive Airway Pressure.
- **EPAP:** Expiratory Positive Airway Pressure.
- **O2:** Oxygen levels.

SaO2 Min/Max

- **SpO2:** Blood oxygen saturation levels (ranging from 50% to 100%).

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Case Report

Sevelamer-induced Stercoral Ulceration in a Patient with End Stage Renal Disease

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Abstract: This is a case describing the sequence of stercoral ulceration leading to fecal impaction and subsequent feculent peritonitis in a patient with end-stage renal disease (ESRD). Analyzing the patient's operative course and hospitalization illustrates the complex nature of ESRD and the associated circumstances leading to this patient's death. Utilizing the patient's imaging and pathology results concludes that medication resins such as sevelamer caused mucosal ulceration in her colon. These results emphasize current literature which describes the potential for gastrointestinal complications with use of sevelamer.

Keywords: sevelamer, colonic ulceration, feculent peritonitis, end-stage renal disease, hyperphosphatemia

1. Introduction

Sevelamer is a commonly prescribed resin-based phosphate binder for patients with end-stage-renal disease (ESRD) receiving dialysis. While it poses the benefit of preventing hyperphosphatemia, chronic use of sevelamer has been consistently linked with gastrointestinal side effects, such as prolonged constipation with the potential for sevelamer crystallization in the gastrointestinal tract [2]. Long term use may be further complicated with heightened risks of mucosal ulceration and an increased risk for bowel perforation. Specifically, stercoral mucosal ulceration refers to pressure-induced necrosis and ulceration of the colonic mucosa due to prolonged fecal impaction, most often in the sigmoid colon. If left untreated, it can progress to colonic perforation and feculent peritonitis. [1]. This is largely due to the mechanism of action of sevelamer and its strong phosphate binding capacity within the gastrointestinal tract. Sevelamer is a non-absorbable polymer that binds phosphate in the gut; however, when it crystallizes, it may deposit in the colonic mucosa. These crystal fragments can induce direct epithelial injury and inflammation, as well as ulceration in vulnerable areas of the bowel [5]. Consequently, many patients with ESRD on dialysis have elevated risks of gastrointestinal adverse effects due to polypharmacy and heightened vulnerability to mucosal complications. Although the mechanism of mucosal injury induced by sevelamer is unclear, it is understood that sevelamer crystallization may deposit into intestinal epithelial cells and subsequently cause cell damage and death [3]. As a result, patients may experience greater risks of bowel obstruction and perforation.

Although conventional management of ESRD may involve the use of sevelamer, it should be judiciously prescribed in patients with history of constipation and/or prior abdominal surgeries in order to decrease the risk of gastrointestinal impairments and potential perforation [7]. Moreover, the presence of multiple comorbidities in patients with ESRD should warrant careful consideration with close monitoring of patients on medication resins. Thus, it is

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crucial to recognize the risk factors which would caution the use of sevelamer in patients with ESRD. Furthermore, identification of these severe adverse effects of sevelamer should be recognized and appropriately considered when prescribing this medication.

2. Case Presentation

Here, we discuss a case of a 66-year-old female with a complex prior medical history including chronic kidney disease, systemic lupus erythematous, hyperlipidemia, hypertension, coronary atherosclerotic disease, congestive heart failure, seizure disorder, and failed renal transplant who presented to the emergency department with abdominal pain. The patient was found to have a perforation of the sigmoid colon with feculent peritonitis. Despite initial efforts of conservative treatment with antibiotics, the patient's condition had rapidly deteriorated and necessitated emergent operative intervention.

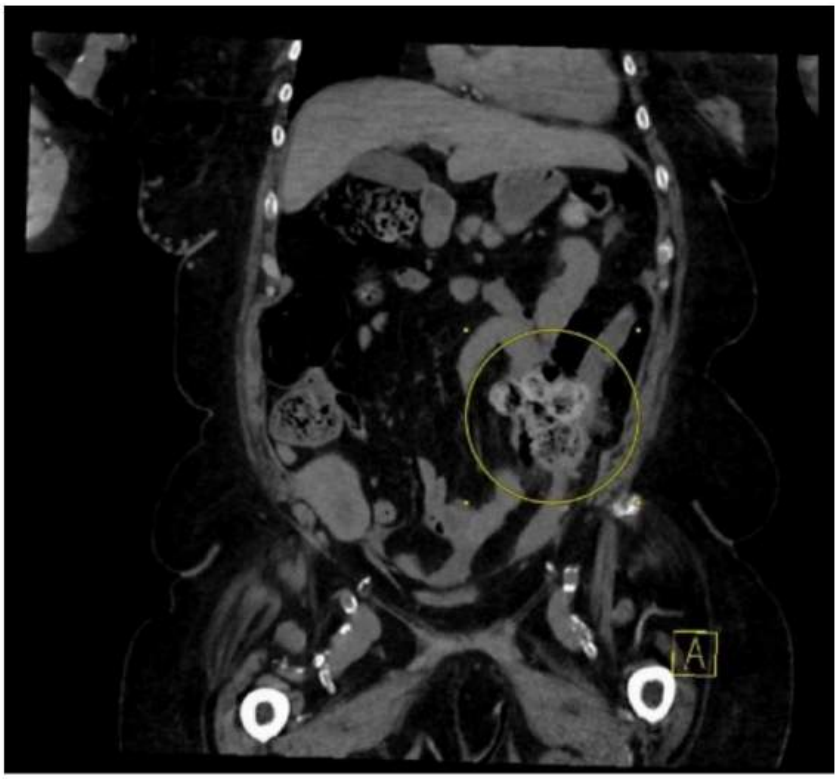

Figure 1. This coronal section of the CT of abdomen and pelvis demonstrates the patient's worsening condition with significant inflammatory changes of the descending and sigmoid colon.

The patient initially underwent exploratory laparotomy with sigmoid colon resection. Multiple stool balls were extracted within her sigmoid colon and significant adhesions between rectosigmoid colon and surrounding soft tissue were lysed using blunt dissection and electrocautery. An Abthera VAC was placed for temporary abdominal closure. Her condition continued to decline and required observation in the intensive care unit with mechanical ventilation as well as use of pressors. She was admitted to the ICU for management of septic shock. On postoperative day two, she was taken back to the OR for revision with mobilization of the descending colon and creation of an end colostomy. She remained in the ICU with mechanical ventilation following surgery and remained on pressors due to hemodynamic instability.

Subsequently, specimens from the patient were collected and resulted by surgical pathology, which demonstrated sevelamer in association with mucosal

injury and perforation. Specifically, there were fragments of resin-material noted within the pathology report. These findings were consistent with sevelamer crystallization and particle deposition. It was also noted that the area of bowel perforation demonstrated neutrophilic inflammation and fat necrosis, which may further support the hypothesized mechanism of sevelamer-induced mucosal injury.

On postoperative day five, the patient's condition continued to worsen, and CT imaging with contrast demonstrated hemoperitoneum, and ischemic colitis of the transverse colon. This required a return to the OR for transverse colectomy and revision to an ascending colostomy in the right upper quadrant. She continued to be closely monitored within the ICU and treated by the critical care team for multifactorial distributive shock. Despite continued efforts by multidisciplinary teams, the patient further declined and required pressure support ventilation as well as intermittent use of pressors. She became unstable postoperatively to tracheostomy placement on post-operative day 21. The patient was transitioned to palliative care following a goals-of care discussion with her family, and she eventually passed away after a 21-day hospital course.

3. Discussion

This patient's chronic kidney disease was evidently compounded by stercoral ulcer formation which eventually led to colonic perforation and ischemic colitis. Stercoral ulceration and fecal impaction are rare but significant drawbacks to using phosphate binders such as sevelamer in patients with ESRD.

This patient had been prescribed sevelamer many years prior to this complication and was taking the standard dose of 800mg orally three times daily for over five years to prevent hyperphosphatemia. However, she was taking multiple other medications for her comorbidities that may have also interacted with sevelamer. In addition, she had also been prescribed lanthanum carbonate, which is a metal-based phosphate binder similar to sevelamer. Although she did not have documented previous bowel obstruction (which would have contraindicated her use of sevelamer), she did have prior abdominal surgical history which further exacerbated her risks for gastrointestinal difficulties. In addition, she had multiple comorbid conditions (autoimmune disease, congestive heart failure, hyperlipidemia, and hypertension) which heightened her risk for mucosal injury [7]. Therefore, these potential complications should be considered when deciding to manage hyperphosphatemia with resin vs. metal-based binders in order to help prevent the development of mucosal injury with sevelamer [5].

Statistical studies have shown that approximately 40-60% of ESRD patients require phosphate binders, with sevelamer being one of the most prescribed agents due to its non-calcium, non-metallic composition. However, the incidence of gastrointestinal complications, such as mucosal injury and bowel perforation, is reported to be much higher in patients using sevelamer over extended periods, especially in those with a history of gastrointestinal disease or prior abdominal surgeries [6]. It is important to recognize that gastrointestinal side effects, including severe complications like stercoral ulceration, are significantly more frequent in patients who have additional risk factors, such as chronic constipation, previous abdominal surgeries, or the use of constipating medication.

Several case reports—approximately 10 published over the past two decades—have described sevelamer-induced mucosal injury progressing to colonic perforation. Although rare, the cumulative evidence highlights a recognizable pattern of gastrointestinal injury associated with sevelamer crystal deposition and subsequent bowel wall compromise. These reports often involve patients with chronic kidney disease or ESRD, reflecting similar patient demographics to our patient. Notably, mucosal abnormalities have been observed throughout both the upper and lower gastrointestinal tracts, with variable clinical manifestations [3]. The most frequently reported presentation is gastrointestinal

bleeding, which in some cases progresses to peritonitis or acute abdomen requiring surgical intervention, as demonstrated in our patient [5]. Additionally, prior studies suggest that underdiagnosis of sevelamer-induced gastrointestinal disease may be partially attributed to its common side effects—particularly chronic constipation and reduced gut motility—which can mask more serious underlying mucosal damage [7]. These findings reinforce the importance of maintaining a high index of suspicion for sevelamer-related complications in symptomatic patients, especially in patients with complex medical histories and multiple comorbidities.

Mucosal injury with colonic perforation secondary to sevelamer use in end-stage-renal disease certainly raises concern for clinical outcomes for complex cases as described above. The various risk factors within this patient's case had also likely contributed to her overall clinical course and placed her at a greater risk for gastrointestinal complications. Recognition of this potential consequence is imperative for future management of patients with ESRD on sevelamer and highlights the importance of clinical guidance when managing complex patients with multiple comorbidities. This case is unique in its severity and rapid progression, complicated by pre-existing immunosuppression, prior abdominal surgeries, and multifactorial shock. To our knowledge, few cases describe such extensive colonic damage with both stercoral ulceration and ischemic colitis linked to sevelamer deposition.

Funding: This research received no external funding

Institutional Review Board Statement: Ethical review and approval were waived for this study due to exemption status of case reports.

Informed Consent Statement: Informed consent was waived in this case because the patient died before the opportunity to obtain consent could be presented, rendering the process impractical due to their deceased status.

Conflicts of Interest: The authors declare no conflicts of interest.

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CLINICAL PEARLS

Clinical Pearls

Bridging gaps in healthcare: The journey of a student-run free clinic in Puerto Rico

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Keywords: student-run free clinic; preclinical; medical education; volunteers; health disparities; clinical skills; interprofessional

1. Clinical Pearl

Founding of Clínica del Sur: Disparities in healthcare access and affordability exacerbate the constraints presented by a complex socioeconomic landscape in Puerto Rico (PR). According to the U.S. Census Bureau, the median household income in PR is $24,112, and 41.7% of people live in poverty on the island [1]. While the U.S. Census Bureau states that just 5.1% of people in PR do not have healthcare coverage, the healthcare system in PR struggles with employee shortages and lack of federal funding [2]. This makes healthcare less accessible despite Puerto Ricans having health insurance. As the elderly population continues to grow to the point that the percentage of elderly people in PR is among the top ten globally, challenges arise in accessing healthcare with family members migrating to the U.S. [1].

In 2019, medical students at Ponce Health Sciences University (PHSU) sought out to address these healthcare disparities. Despite significant earthquakes and the COVID-19 pandemic, the Student-Run Free Clinic (SRFC) Clínica del Sur was born in 2022. The clinic serves as a reliable resource for underserved populations and an opportunity for preclinical health sciences students to gain clinical experience and conduct research. Since its founding, Clínica del Sur has been run by students in medicine, psychology, nursing, and public health. Since then, the clinic has expanded to offer dermatology, dental, and neurology services. Clínica del Sur strives to provide comprehensive care in these areas to best serve the needs of its community similar to the Student-Run Clinic of University of Texas Río Grande Valley School of Medicine, which treats patients who do not have access to primary care [3]. Other clinics such as Qlinic, an LGBTQIA+ clinic at Cornell University, focus on one area of healthcare such as behavioral therapy in mental healthcare [4].

As a member of the National Association of Free and Charitable Clinics (NAFC), Clínica del Sur has been funded by the following organizations: Americares, Direct Relief, Brother's Brother Foundation,

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Fundación Intellectus, and Heart to Heart Foundation. Through this membership, the clinic has also received a grant from CeraVe, from which the Sun Protection Campaign was launched to raise awareness and provide education on sun protection. Equipment has been acquired through the grant to improve the clinic's dermatology services. For example, many patients are lost to follow-up due to the complicated structure of the island's healthcare system. To relieve patients of this burden, the clinic acquired a camera to capture photos of areas of concern on the skin so that patients have the necessary information in their file for their referral for continued cost-free care. The clinic is also a member of the Society of Student-Run Free Clinics (SSRFC), through which it hopes to gain funding and networking opportunities.

Walkthrough of the Clinic: Patients enter the community center's doors, greeted by volunteers at a registration table. Nursing students record vital signs and any medical or social concerns. Then, patients visit the next station where a medical student documents their patient history.

Patients then visit stations such as public health, psychology, dental, dermatology, and neurology. Public health offers educational tables on topics including identification of mosquitoes carrying Dengue virus. Psychology, dental, dermatology, and neurology offer services based on patients' needs, including screening for depression, dental evaluations, skin tag removal, or stroke prevention information, respectively. Referrals are made to psychiatry, psychology, social work, and to patients' primary care physicians.

Lastly, public health students complete a health and satisfaction survey on REDCap with patients. The information collected serves as a database for current and future research to assess community needs and clinic quality.

Additional services are coordinated when necessary. The main food bank, Banco de Alimentos de PR, provides groceries for patients. Clínica del Sur participates in the Back-to-School Health Fair to provide dental certificates and vaccinations to school-aged children. PR's more remote island, Vieques, has less resources than the mainland. To address this, the clinic travels to Vieques for house calls and health fairs. The clinic utilizes a mobile unit when visiting remote areas on the mainland. Figure 1 depicts the framework of Clínica del Sur. Appendix A demonstrates the range of services. Appendix B highlights the geographical outreach

2. Tables, Figures, and Schemes

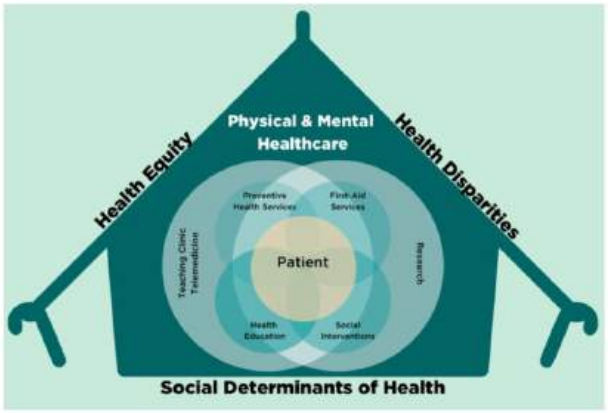

Figure 1. Clínica del Sur Framework. Clínica del Sur aims to address comprehensive health needs by providing physical and mental health services, which include prevention, first-aid, health education, and social interventions. The clinic also includes teaching to facilitate interprofessional education and offers space for nested research studies.

Challenges and future steps: Students involved in clinic research present at conferences such as the 2024 SSRFC Conference and the NAFC 2024 Charitable Healthcare Symposium. By participating in these events, Clínica del Sur acquires knowledge and connections that strengthen its quality and longevity. The main concern for the clinic's longevity is funding, as the purpose of Clínica del Sur is to provide free healthcare services for the long-term. A sustainable, reliable financial foundation is needed for that to remain a reality. Our goal is to secure more substantial and long-term funding. To achieve this, we are in the process of applying for grants that provide annual funding.

Another challenge faced is that patients' health records are currently documented on paper, which is time-consuming to update. Finding an affordable electronic health record has proven difficult, but Inmediata was gracious in granting the clinic use of their system.

Future improvements include educational sessions led by public health students to address community needs, including prevention of sexually transmitted infections, diabetes, heart disease, etc. We plan to welcome doctoral students in Public Health, Psychology, and Biomedical Sciences to develop and implement their thesis projects at the clinic. We plan to have social workers at the clinic, replacing our current referral system. Clínica del Sur is the first interaction many students have with patients, thus more structured training for student volunteers prior to their first clinic is being developed. To expand the clinic's interdisciplinary aspect, we plan to adjust the patient history documentation station to include pairs of psychology and medical students.

Clínica del Sur serves PR with the intention of providing free, accessible healthcare and invaluable learning opportunities for students. We continue addressing PR's healthcare disparities while also bridging the gap between academic learning and real-world practice with the intention of molding students into compassionate, community-centered leaders in healthcare.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: Clínica del Sur Framework.

Author Contributions: Conceptualization, L.D. and B.H.; methodology, B.H.; investigation, LD. and B.H.; resources, L.D.; writing—original draft preparation, B.H.; writing—review and editing, L.D. and B.H.; visualization, B.H.; supervision, L.D.; project administration, L.D.; funding acquisition, L.D. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

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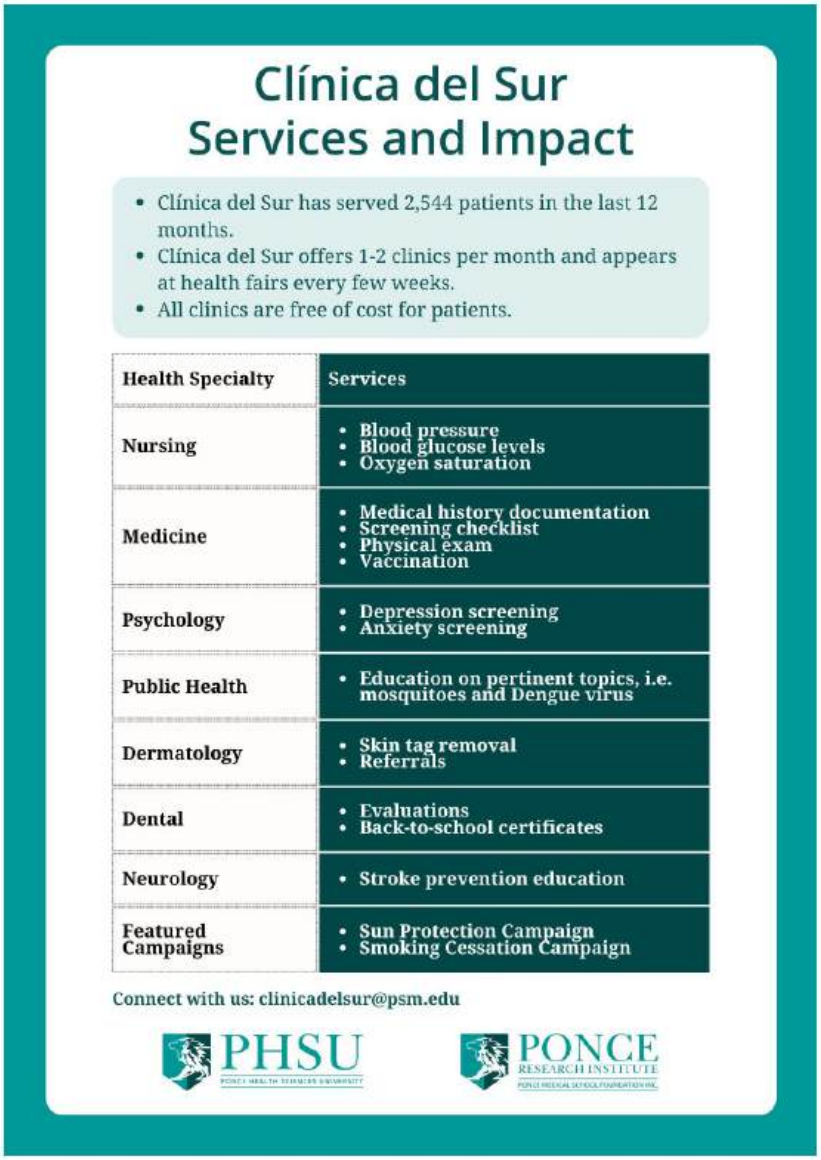
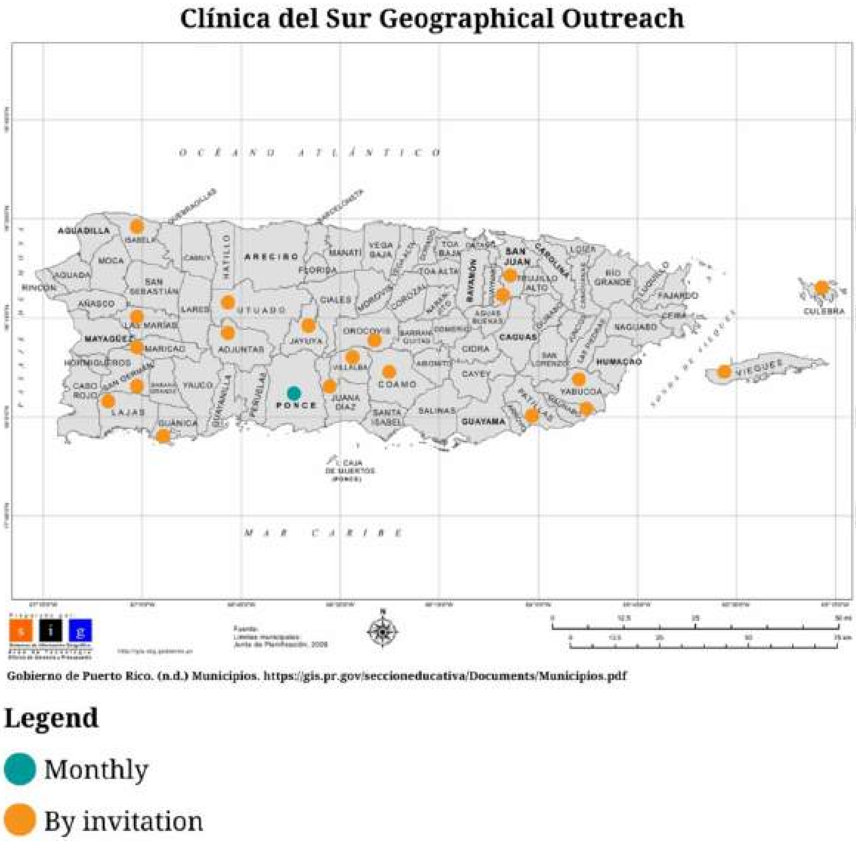
Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A


Clínica del Sur
Services and Impact
• Clínica del Sur has served 2,544 patients in the last 12 months.
• Clínica del Sur offers 1-2 clinics per month and appears at health fairs every few weeks.
• All clinics are free of cost for patients.
Health Specialty
Services
Nursing
• Blood pressure
• Blood glucose levels
• Oxygen saturation
Medicine
• Medical history documentation
• Screening checklist
• Physical exam
• Vaccination
Psychology
• Depression screening
• Anxiety screening
Public Health
• Education on pertinent topics, i.e. mosquitoes and Dengue virus
Dermatology
• Skin tag removal
• Referrals
Dental
• Evaluations
• Back-to-school certificates
Neurology
• Stroke prevention education
Featured Campaigns
• Sun Protection Campaign
• Smoking Cessation Campaign
Connect with us: clinicadelsur@psm.edu
PHSU
PONCE RESEARCH INSTITUTE

Appendix B



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LETTERS TO THE EDITOR

Letter to the Editor

Generative AI as a Tool for Enhancing ESL Students' Understanding

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Wilfredo De Jesús-Rojas, MD
Ponce Health Sciences University Scientific Journal
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Dear Editor-in-Chief,

As former ESL (English as a Second Language) students who have pursued science, technology, engineering and mathematics (STEM) fields and current researchers and instructors, we intimately understand the challenges faced by students adapting not just to a new language, but to the complex context in which that language is used in academic settings. Our experiences have given us unique insights into the difficulties ESL students encounter in higher education, particularly in STEM disciplines.

Research aligns with our personal observations. ESL students often struggle with academic language proficiency, which can severely impact their ability to understand complex materials and engage in academic discourse [1]. These students are also more likely to face higher attrition rates [2], with studies indicating that they are more likely to experience academic probation or drop out compared to their native English-speaking peers [3]. ESL students often encounter significant obstacles in accessing and understanding academic resources that are primarily available in English, which can impede their ability to fully engage with the material.

To illustrate the challenges and potential solutions, we would like to share an experience from Nelson's academic journey that led us to appreciate the potential of generative AI in ESL students' education. During his graduate studies, Nelson was enrolled in a rigorous Real Analysis course. Despite his best efforts, he found himself struggling to grasp the material. In a meeting with his then mentor, Prof. Doug Moupasiri, Nelson confessed his difficulties. Prof. Moupasiri asked a simple yet profound question: "*What other books on the subject have you read?*" When Nelson admitted that he had only been using the assigned textbook, Prof. Moupasiri encouraged him to explore other authors' works. This advice—to seek out different perspectives—was a turning point in Nelson's academic career. By finding an author whose style resonated with him, he was able to understand concepts that had previously eluded him. This experience made us realize that sometimes, the issue is not with the subject itself but with how it is presented.

This is where we see a tremendous opportunity for generative AI tools to support students, particularly ESL students, in their learning journey. ESL students often benefit from different learning approaches, and findings show that they have positive perceptions of AI-based learning tools, appreciating their personalized learning paths and time-saving advantages [4]. As evidenced in recent studies [5],

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AI technologies are already being successfully used in medical education to provide real-time feedback on quizzes, assist in anatomy learning, and support the recognition and diagnosis of medical images. Additionally, a study performed in a medical school in Puerto Rico showed that integrating a course aimed to bridge the gaps in AI knowledge among participants resulted in more positive perceptions of AI. However, it also revealed a lack of practical experience with AI applications, emphasizing the need for better integration of AI into educational programs [6].

Initiatives aimed specifically at Hispanic students are demonstrating the value of incorporating AI literacy into their education, helping them critically evaluate and effectively use AI technologies across different contexts [7]. By equipping students with essential AI competencies, these programs foster not only academic success but also readiness for AI-rich environments at home and in the workplace. We believe extending such support to ESL students can enhance equitable access to education by providing adaptive and personalized learning paths that cater to specific language needs. Just as different authors can present the same subject in varying ways, generative AI can offer students alternative explanations, analogies, and examples that align more closely with their individual learning styles. This ability to reshape content makes generative AI particularly powerful for students at institutions in Puerto Rico and elsewhere, allowing them to bridge gaps in understanding through tailored explanations in their native language or more accessible rephrasing, ultimately making challenging subjects more comprehensible.

Generative AI is not just a tool for information retrieval but a companion in the learning process, helping students navigate complex subjects with a personalized approach that traditional methods may not always provide. The greatest value of generative AI as a tutor lies in its ability to customize learning experiences, tailoring them to fit the context and background of each student. As we continue to integrate AI into the educational landscape, it is our hope that we can leverage these tools to not only enhance learning but also to empower students to overcome the challenges that come with language barriers and different learning styles. Just as Nelson's mentor's advice reshaped his educational path, we believe encouraging the correct use of generative AI can play a similar role for many students, directly impacting academic outcomes.

We urge educators and administrators to consider the potential of generative AI as a powerful tool for addressing the unique challenges faced by ESL students and those from diverse backgrounds. By embracing this technology, we can create a more inclusive and effective learning environment that supports all students in reaching their full potential.

Sincerely,

Nelson Colón Vargas, PhD and Marcos J. Ramos-Benítez, PhD

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