IN THE NAME OF ALLĀH,
THE MOST GRACIOUS, THE MOST MERCIFUL

JOURNAL OF

KING ABDULAZIZ UNIVERSITY

MEDICAL SCIENCES

VOLUME 31 NUMBER 2

2024 A.D. / 1446 A.H.

SCIENTIFIC PUBLISHING CENTRE
King Abdulaziz University
P.O. Box 80200, Jeddah 21589
Saudi Arabia

Journal of
KING ABDULAZIZ UNIVERSITY
MEDICAL SCIENCES

Volume 31, Number 2, DecemberJune 2024 AD/1446 AH ISSN 1319- www.jkaumedsci.org.sa

EDITORIAL BOARD

EDITORIAL STAFF

Editor-in-Chief
Journal of King Abdulaziz University – Medical Sciences
King Fahad Medical Research Center, King Abdulaziz University
P.O. Box 80216 , Jeddah 21589, Saudi Arabia
T:+966-012-695-2000 Ext: 20222 ; F: 966-012-695-2000 Ext:25651

Journal of
KING ABDULAZIZ UNIVERSITY
MEDICAL SCIENCES

Volume 31, Number 2, December 2024 AD/1446 AH ISSN 1319-1004 www.jkaumedsci.org.sa

contents

ORIGINAL ARTICLES

REVIEW ARTICLES

ORIGINAL ARTICLE

The Prevalence Regarding the Use of Glucagon-like Peptide-1 (GLP-1) Agonists as an Obesity Treatment among Adult Patients at King Abdulaziz University Medical Services Center, Jeddah, Saudi Arabia: A Descriptive Cross-Sectional Study

Mohammed A. Alsieni[1], MD, PhD, Raghad B. Babader[2], Shahad A. Kenany[2], Shahad A. Abdulgader[2], Raneem F. Alafif[2], Atheer A. Alahmadi[2], Albatool A. Aloufi[2], Eman A. Fallatah[2], Rawyah A. AlQahtani[2], Alyaa M. Izaldin[2]

[1]Department of Pharmacology, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia
[2]Medical Student, King Abdulaziz University, Jeddah, Saudi Arabia

Correspondence
Raghad B. Babader
King Abdulaziz University
P.O. Box 80205, Jeddah 21589
Kingdom of Saudi Arabia
e-M: bader2002@gmail.com

Submission: 20 Jun. 2024
Accepted: 15 Nov. 2024

Citation
Alsieni MA, Babader RB, Kenany SA, Abdulgader SA, Alafif RF, Alahmadi AA, Aloufi AA, Fallatah EA, AlQahtani RA, Izaldin AM. The prevalence regarding the use of glucagon-like peptide-1 (GLP-1) agonists as an obesity treatment among adult patients at King Abdulaziz University Medical Services Center, Jeddah, Saudi Arabia: A descriptive cross-sectional study. JKAU Med Sci 2024; 31(2): 1-12. DOI: 10.4197/Med.31-2.1

Keywords
Glucagon-like Peptide-1 agonists, Obesity treatment, Adult patients, King Abdulaziz University Medical Services Center, Jeddah, Cross-sectional study

Abstract
Background: Glucagon-like peptide-1 (GLP-1) receptor agonists effectively achieve and sustain body weight reduction in individuals with obesity. However, there are few studies on the safety and tolerability of GLP-1 agonists in the Saudi Arabian population with obesity.
Objectives: To assess the prevalence of GLP-1 agonists used for obesity treatment and the associated adverse effects among adult populations at King Abdulaziz University Medical Services Center, Saudi Arabia.
Methods: A cross-sectional study was conducted at King Abdulaziz University Medical Services Center with 188 participants, all above 18 years of age with Body Mass Index (BMI) ≥ 27, using GLP-1 agonists. A validated questionnaire was used to collect data on participants' demographics, chronic diseases, smoking habits, weight, height, family support for weight loss, cause of obesity, weight loss attempts, GLP-1 agonist usage patterns, negative side effects of GLP-1 agonist use, and patient satisfaction.
Results: The mean age of participants was 46.24 ± 12.78 years, with 56.9% being male and 85.6% of Saudi nationality. The mean BMI was 34.78 ± 5.3 kg/m^2, and 80.9% were classified as obese. Semaglutide was the most commonly used GLP-1 agonist (66%). For 86.2% of participants, the medication was prescribed by a physician, with a mean medication use duration of 12.43 ± 14.73 months. The mean weight loss during treatment was 10.11 ± 6.53 kg, and 70.2% had negative side effects with nausea (51.1%) being the most common. Of the participants, 65.5% were satisfied with the GLP-1 therapy outcome and 78.7% would recommend its use to others. The incidence of adverse effects was significantly higher among those using GLP-1 agonists for diabetes and weight reduction.
Conclusion: GLP-1 receptor agonists are promising agents for obesity treatment, with a mean weight loss of approximately 10 kg among participants. Despite the side effects, most patients were satisfied with the drug and would recommend its use to others.

INTRODUCTION

Saudi Arabia has experienced increasing westernization over the past few decades, leading to a surge in obesity and overweight cases. According to estimations by the World Health Organization (WHO), the Kingdom of Saudi Arabia (KSA) has an overall obesity prevalence of 33.7% and an overweight prevalence of 68.2%. The high prevalence of obesity in Saudi society suggests that preventive efforts are either lacking or ineffective[1].

Being overweight or obese is a biological risk factor for several significant health disorders including diabetes, hypertension, and cardiovascular diseases. In addition, being overweight has a negative impact on a person's physical and mental health, lifestyle, and finances due to reduced productivity, increased disability, higher healthcare costs, and shorter life spans[2].

This challenge needs to be resolved as Saudi Arabia is undergoing a significant economic transition as part of Vision 2030 to boost the nation's human capital, guarantee quicker economic growth, and build a workforce that is healthy and prepared for a productive future[2].

A class of medications known as glucagon-like peptide-1 (GLP-1) agonists are used to treat persons with type 2 diabetes[3]. The medications in this class include exenatide, lixisenatide, liraglutide, albiglutide, dulaglutide, and semaglutide. According to the American Diabetes Association, while metformin remains the primary treatment for type 2 diabetes, GLP-1 agonists are recommended for patients with chronic renal disease, atherosclerosis, heart failure, or elevated hemoglobin A1C (HbA1C) levels, 1.5% or higher[3].

The FDA has approved semaglutide and high-dose liraglutide as pharmacological therapies for obesity; patients who are overweight and have concurrent health issues may be urged to use these drugs[3]. These drugs are currently under assessment for their potential to reduce the incidence of obesity. Moreover, semaglutide does not require dose adjustments and is safe for use in adults and older individuals with hepatic or renal impairments[4].

GLP-1 receptor agonists reduce hunger and appetite, increase feelings of fullness after meals, and delay stomach emptying. The most common adverse effects of the medication are nausea, vomiting, and diarrhea, which often start within the first few weeks of treatment and subsequently subside[5].

According to a study by Ard et al. (2021), GLP-1 receptor agonists are a highly effective and well-tolerated form of treatment that can help individuals with obesity achieve and sustain 5–10% body weight reductions[5].

Barritt AS 4th, et al. (2022) showed that liraglutide and semaglutide, two GLP-1 receptor agonists, were associated with a clinically substantial and long-lasting reduction in body weight in individuals who were overweight or obese[6]. The same effectiveness of semaglutide was observed in a previous double-blind, randomized controlled trial, where the mean change in body weight from baseline to week 68 was -14.9%[7].

Liraglutide users often lose 4–7 kg after taking the medication. Semaglutide has a more pronounced effect on weight loss, resulting in a mean weight reduction of 9–16 kg. These outcomes have contributed to the regulatory approval of these medications for treating obesity, even in non-diabetic individuals[8].

According to the Saudi Arabian Society for Metabolic and Bariatric Surgery (SASMBS) guidelines, GLP-1 agonists, including liraglutide, can be used to treat obesity and reduce the number of morbidly obese individuals in Saudi Arabia[9]. In addition, GLP-1 agonists are a great substitute for invasive procedures such as liposuction and bariatric surgery[10,11].

However, there are few studies on the safety and tolerability of GLP-1 agonists in the Saudi Arabian population with obesity. Thus, this study aimed to assess the prevalence of GLP-1 agonists used for obesity treatment and their associated adverse effects among the adult population in Jeddah, Saudi Arabia.

SUBJECTS AND METHODS

Study design, setting, and timeline: This cross-sectional study was conducted at King Abdulaziz University Medical Services Center, Jeddah, Saudi Arabia, from January 2023 to April 2024.

Study participants: The study included adults in Jeddah who were above 18 years old, with a BMI of 27 kg/m^2 or above, who were using GLP-1 agonists for weight loss, and who were willing to participate.

Participants under 18 years, pregnant women, and adults unwilling to provide informed consent were excluded.

Sample size: A total coverage sample of approximately 188 individuals was obtained during the study period, and all adults who met the inclusion criteria were included. A total of 873 patients who had received the medication were contacted; however, we excluded 685 patients, of whom 550 did not respond to either WhatsApp or phone calls, 31 refused to participate in the study, and 60 had an incorrect phone number as a result of incorrect documentation or had a BMI below 27 kg/m^2. Patients under 18 years were excluded from the study.

Data collection: Data were collected through phone calls and face-to-face surveys (Google Forms). To our knowledge there was no validated questionnaire relevant to our study; therefore, we administered our own questionnaire, which contained a number of multiple-choice questions in both English and Arabic. All questions were designed to assess the prevalence of GLP-1 agonists used for obesity treatment among adults in Jeddah. The questionnaire was designed to collect personal data by asking about sex, age, nationality, marital status, occupation, monthly income, weight, height, obesity causes, chronic diseases, and smoking status.

The questionnaire included questions to assess family support regarding the participants' efforts to lose weight, unsuccessful previous weight loss attempts, the name of the drug that they took for weight loss and the reason for its use, who recommended the medication when they started using this medication, duration of drug use, administration methods, frequency, and number of kilograms lost during the treatment period. Moreover, it included questions assessing the negative effects of taking these medications for weight loss, medication type, and patient satisfaction with the results.

The research instrument was validated by a medical expert through a pilot study involving 20 participants (patients who take GLP-1 in King Abdulaziz University Medical Services Center, Jeddah, Saudi Arabia). Data were transferred to an Excel sheet to share with the expert; then, data was coded using SPSS. The evaluation considered the clarity of the questions, the time required to answer the questions, and alignment with study objectives; the questionnaire was then modified accordingly. Cronbach's alpha value for assessing reliability was 8.3%.

Ethical considerations: Ethical approval for the study was obtained from the Research Ethics Committee of King Abdulaziz University Medical Services Center, Jeddah, Saudi Arabia. Informed consent was obtained from all participants before their participation in the study. The participants were provided with detailed information regarding the purpose of the study.

To ensure confidentiality, we did not use the participants' names; instead, we assigned unique codes to each participant. This coding system allowed us to collect and analyze data without revealing any identifying information. Data were securely stored in password-protected files accessible only to authorized research personnel.

Data analysis: Statistical analysis was performed using the SPSS application version 26. To investigate the association between the variables, the Chi-square test ($\chi 2$) was used to analyze qualitative data expressed as numbers and percentages. Quantitative variables were expressed as mean ± standard deviation (Mean ± SD). Multivariate logistic regression analysis was performed to assess factors associated with the development of negative effects from weight loss medications. Odds ratio (OR) with a 95% confidence interval (CI) was calculated, and a *p*-value of < 0.05 was considered statistically significant.

RESULTS

The mean BMI of the study participants was 34.78 ± 5.3; of these, 36 (19.1%) were classified as overweight, and 152 (80.9%) as obese. The mean age was 46.24 ± 12.78 years, 56.9% were males, 85.6% held Saudi nationality, and 79.3% were married. In addition, 67.6% were employed, and 28.1% had a monthly income of 10,000 - 19,999 SAR. Forty-two percent of the participants had chronic diseases, with diabetes and hypertension being the most common (58% and 41.5%, respectively). Approximately 26% (26.1%) were current smokers (Table 1).

The majority (84.6%) received support from family members for their weight-loss efforts. The most common causes of obesity were lack of exercise (71.8%) and overeating (52.7%). Approximately 72% (72.9%) had previous unsuccessful weight loss attempts, with diet (65.4%) and exercise (42%) being the most common

Table 1. Distribution of study participants according to their demographic characteristics, chronic diseases, and smoking status (No.: 188).

Variable	No. (%)
Age (Mean ± SD) (years)	46.24 ± 12.78
Gender	
Female	81 (43.1)
Male	107 (56.9)
Nationality	
Non-Saudi	27 (14.4)
Saudi	161 (85.6)
Marital status	
Divorced	10 (5.3)
Married	149 (79.3)
Single	24 (12.8)
Widow	5 (2.7)
Occupation	
Employed	127 (67.6)
Retired	29 (15.4)
Student	13 (6.9)
Unemployed	19 (10.1)
Monthly income	
less than 4000	18 (9.6)
4000-9,999 SAR	17 (9)
10,000-14,999 SAR	36 (19.1)
15,000-19,999 SAR	17 (9)
More than 20,000 SAR	16 (8.5)
I don't have monthly income	17 (9)
I prefer not to answer	67 (35.6)
Do you have any chronic diseases?	
No	109 (58)
Yes	79 (42)
If having a chronic disease, specify:	
Diabetes mellitus (DM)	109 (58)
Hypertension (HT)	78 (41.5)
Thyroid disease	28 (14.9)
Respiratory disease	17 (9)
Migraine or tension headaches	10 (5.3)
Gastrointestinal diseases	22 (11.7)
Cardiac disease	20 (10.6)
Kidney diseases	2 (1.1)
Neurological diseases	9 (4.6)
Anxiety, depression, or any psychological diseases	15 (8)
Others	19 (10.1)
Do you smoke?	
No	121 (64.4)
Ex-smoker	18 (9.6)
Yes	49 (26.1)

SD: standard deviation

methods used to lose weight. The most commonly used GLP-1 agonists were semaglutide (Ozempic or Rybelsus) (66%) and liraglutide (Victoza or Saxenda) (39.4%). More than half (54.8%) of the participants used the medications for both diabetes and weight reduction, and 86.2% reported that a physician made the recommendation. The mean duration of medication use was 12.43 ± 14.73 months. Most participants used injectable forms of the medication, with 60.1% taking it once a week. The mean number of kilograms the participants lost during the treatment period was 10.11 ± 6.53 kg (Table 2).

Table 2. Distribution of study participants based on obesity-related circumstances (No.: 188)

Variable	No. (%)
Do your family members encourage your efforts to lose weight?	
No	29 (15.4)
Yes	159 (84.6)
Cause of obesity	
Lack of exercise	135 (71.8)
Overeating	99 (52.7)
Pregnancy	27 (14.4)
Medications (e.g., cortisone)	19 (10.1)
Psychological factors	43 (22.9)
Others	59 (31.4)
Are there previous weight loss attempts that were unsuccessful?	
No	51 (27.1)
Yes	137 (72.9)
If you have previous weight loss attempts, specify:	
Diet	123 (65.4)
Exercise	79 (42)

Table 2. Distribution of study participants based on obesity-related circumstances (No.: 188).–Continuation

Medication	46 (24.5)
Surgery	9 (4.8)
What is the name of the drug that you take for weight loss?	
Semaglutide (Ozempic or Rybelsus	124 (66)
Liraglutide (Victoza or Saxenda)]	74 (39.4)
Dulaglutide (Trulicity)]	4 (2.1)
Exenatide (Bydureon)]	1 (0.5)
Lixisenatide (Adlyxin)]	1 (0.5)
Tirzepatide(Mounjaro)	3 (1.6)
Why are you using this medication?	
Diabetes and weight reduction	103 (54.8)
Weight reduction	80 (42.6)
Other	5 (2.7)
Who made the medication recommendation?	
Someone on social media	7 (3.7)
Your family	8 (4.3)
Your friends	11 (5.9)
Your physician	162 (86.2)
When did you start using this medication? (Mean ± SD) (Months)	12.43 ± 14.73
Select the administration methods	
Injection	182 (96.8)
Oral	6 (3.2)
How frequently do you use this medication?	
Once daily	70 (37.2)
Twice daily	2 (1.1)
Once weekly	113 (60.1)
Other	3 (1.6)
How many kilograms did you lose during the treatment period? (Mean ± SD) (kg)	10.11 ± 6.53

SD: standard deviation

Figures 1 and 2, and Table 3 illustrate that most participants (70.2%) experienced negative side effects from using the medication, with nausea (51.1%), anorexia (47.9%), and constipation (31.9%) being the most common. For most participants (55.3%), the doctor decided to continue at the same dose regarding the side effects. While most of them (75%) had regular follow-ups with their doctor, only 22.9% followed up with a dietitian regularly. Approximately 78% (78.7%) would recommend GLP-1 agonists to others.

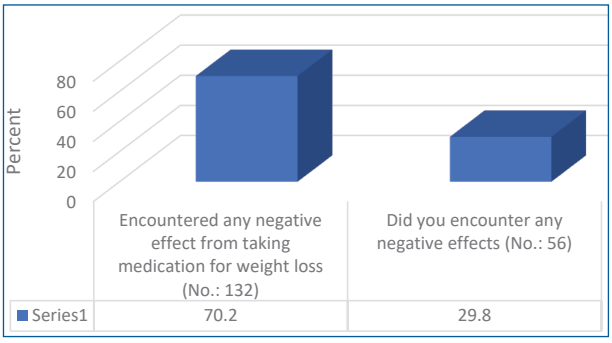


Figure 1. Percentage distribution of the participants according to their encounter of negative effects from weight loss medications (No.: 188).

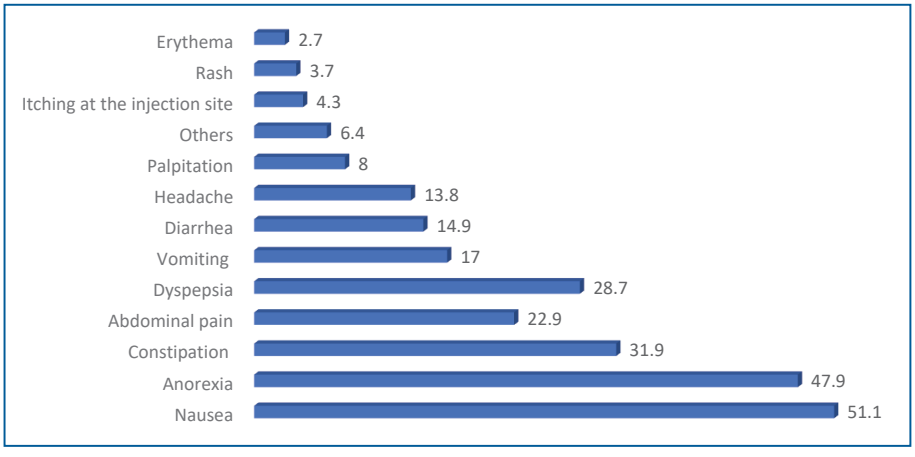


Figure 2. Percentage distribution of the negative effects of weight loss medications (No.: 132)

Table 3. Distribution of participants according to the negative effects of using weight loss medications, regular follow-up with the doctor or dietician regularly, and recommendation of GLP-1 use to others (No.: 188)

Variable	No. (%)
Did you encounter any negative effects from taking medication for weight loss?	
No	56 (29.8)
Yes	132 (70.2)
Mention what was the symptom?	
Anorexia	90 (47.9)
Nausea	96 (51.1)
Dyspepsia	54 (28.7)
Vomiting	32 (17)
Diarrhea	28 (14.9)
Constipation	60 (31.9)
Abdominal pain	43 (22.9)
Palpitation	15 (8)
Rash	7 (3.7)
Erythema	5 (2.7)
Itching at the injection site	8 (4.3)
Headache	26 (13.8)
Others	12 (6.4)
What is a doctor's decision regarding the side effects?	
NA	30 (16)
Change the dose	15 (8)
Complete at the same dose	104 (55.3)
Stop using this medication for some weeks	14 (7.4)
Other	25 (13.3)
Do you follow up with the doctor regularly?	
No	47 (25)
Yes	141 (75)
Do you follow up with a dietitian regularly?	
No	145 (77.1)
Yes	43 (22.9)
Do you recommend others to use GLP-1?	
No	40 (21.3)
Yes	148 (78.7)

Regarding participants' satisfaction with the GLP-1 medication outcome, almost one-third (65.5%) of the participants were satisfied (Figure 3).

Table 4 and Figure 4 show that negative side effects were significantly more common among participants using the medication for both diabetes and weight reduction ($p = < 0.05$). On the other hand, a non-significant relationship was found between developing negative effects and using the drugs, medication duration, who made the medication recommendation, or administration route/frequency ($p > 0.05$).

Multivariate logistic regression analysis assessed the risk factors (independent predictors) for developing negative side effects among the study participants. The analysis observed that none of the studied variables were risk factors (independent predictors) for developing negative effects from the medication ($p > 0.05$) (Table 5).

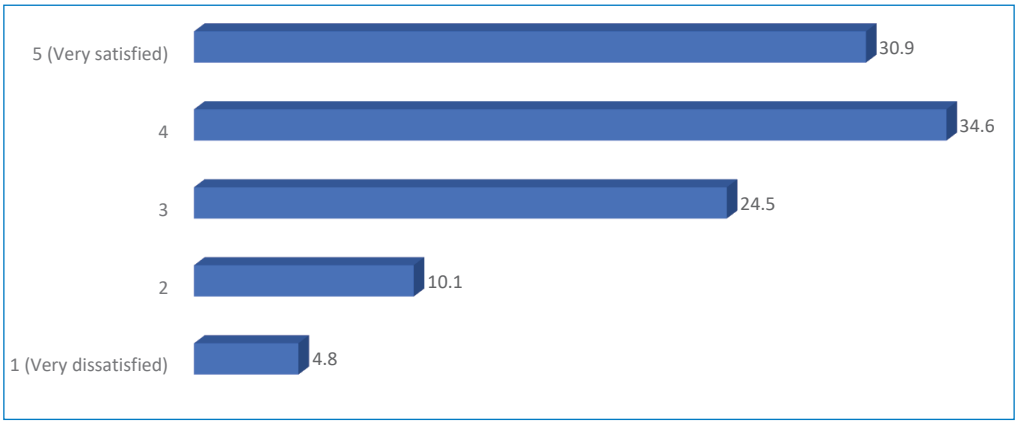


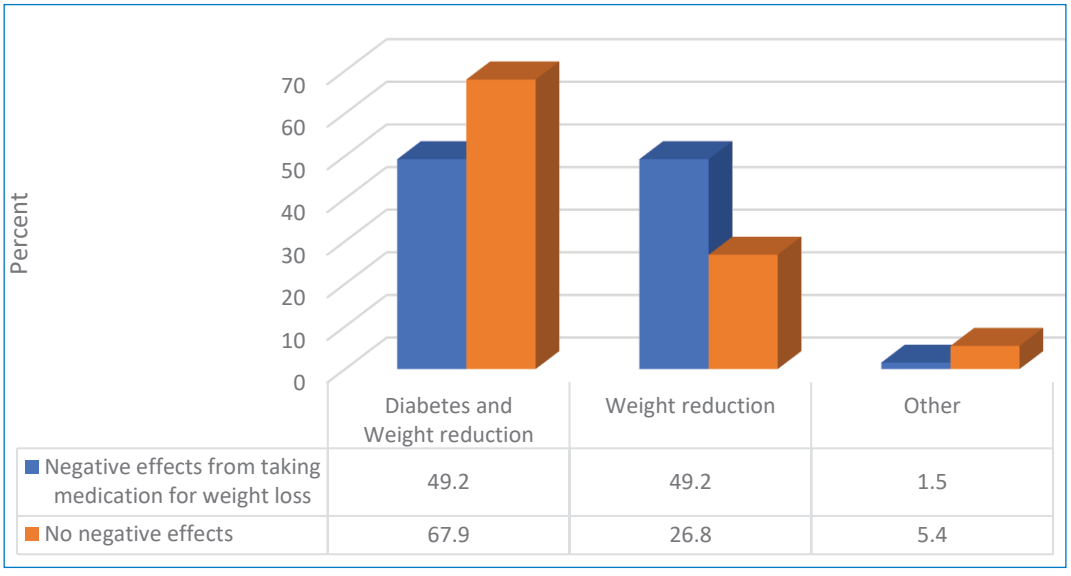
Figure 3. Percentage distribution of the participants' rating (from 1-5) of their satisfaction with the GLP-1 medication outcome (No.: 188)

Table 4. Relationship between developing negative effects from weight loss medications and factors such as medication use, reason for use, duration, source of recommendation, administration route, and frequency (No.: 188)

Variable	Encountered any negative effects from taking medication for weight loss		$\chi 2$	*p*-value
	No	Yes		
	No. (%)	No. (%)		
What is the name of the drug that you take for weight loss?				
Semaglutide (Ozempic or Rybelsus	40 (71.4)	84 (63.6)	1.06	0.302
Liraglutide (Victoza or Saxenda)]	19 (33.9)	55 (41.7)	0.98	0.321
Dulaglutide (Trulicity)]	2 (3.6)	2 (1.5)	0.79	0.372
Exenatide (Bydureon)]	0 (0.0)	1 (0.8)	0.42	0.514
Lixisenatide (Adlyxin)]	0 (0.0)	1 (0.8)	0.42	0.514
Tirzepatide(Mounjaro)	1 (1.8)	2 (1.5)	0.01	0.892
Why are you using this medication?				
Diabetes and Weight reduction	38 (67.9)	65 (49.2)	9.32	0.009
Weight reduction	15 (26.8)	65 (49.2)		
Other	3 (5.4)	2 (1.5)		
Who made the medication recommendation?				
Someone on social media	3 (5.4)	4 (3)	1.94	0.584
Your family	1 (1.8)	7 (5.3)		
Your friends	4 (7.1)	7 (5.3)		
Your physician	48 (85.7)	114 (85.4)		
When did you start using this medication? (Mean ± SD) (Months)	15.18 ± 18.2	11.24 1± 2.86	1.56	1.45
Select the administration methods?	53 (94.6)	129 (97.7)	1.21	0.271
Injection	3 (5.4)	3 (2.3)		
Oral				
How frequently do you use this medication?				
Once daily	18 (32.1)	52 (39.4)	2.97	0.396
Twice daily	1 (1.8)	1 (0.8)		
Once weekly	35 (62.5)	78 (59.1)		
Other	2 (3.6)	1 (0.8)		

Table 5. Multivariate logistic regression analysis of risk factors associated with developing negative effects from weight loss medications

Variable	B	Wald	*p*-value	Odds Ratio (CI:95%)
What is the name of the drug that you take for weight loss?				
Semaglutide (Ozempic or Rybelsus	0.14	0.04	0.834	0.86 (0.21-3.46)
Liraglutide (Victoza or Saxenda)]	0.23	0.17	0.675	0.27 (0.41-1.34)
Dulaglutide (Trulicity)]	0.35	0.08	0.778	0.42 (0.12-1.98)
Exenatide (Bydureon)]	0.15	1.08	0.265	0.16 (0.34-1.65)
Lixisenatide (Adlyxin)]	0.17	0.13	0.176	0.54 (0.13-1.99)
Tirzepatide(Mounjaro)	0.67	1.09	0.451	0.61 (0.91-2.67)
Why are you using this medication?	0.83	3.04	0.081	2.29 (0.9-5.84)
Who made the medication recommendation?	0.18	0.57	0.448	1.2 (0.74-1.97)
When did you start using this medication? (Mean ± SD) (Months)	0.01	1.14	0.285	0.98 (0.96-1.01)
Administration methods	0.67	0.18	0.63	0.96 (0.18-1.39)
How frequently do you use this medication?	0.16	0.23	0.63	1.17 (0.6-2.28)



N.B.: ($\chi 2 = 32$, p-value = 0.009)

Figure 4. Relationship between developing negative effects from medication use and the reason for medication use (No.: 188)

DISCUSSION

This study aimed to determine the prevalence of GLP-1 agonist use for obesity treatment among patients at King Abdulaziz University Medical Services Center in Saudi Arabia and its associated adverse effects.

A multimodal approach to obesity treatment was essential for patients because obesity is considered a chronic and relapsing disease. Anti-obesity drugs are valuable therapeutic agents for managing

obesity[2,3]. Our findings shed light on the importance of participants' health and demographic traits. Among the study population, 19.1% were classified as overweight and 80.9% as obese. These results indicated a considerable burden of excess weight in the group under investigation.

The mechanism of action of GLP-1 agonists and their receptor activity, particularly their non-glycemic effects, and advantages in treating comorbidities associated with insulin resistance, were the rationale behind their use for obesity[12,3]. They play a major role in weight reduction through their effects on gastric emptying time, suppression of inappropriate post-meal glucagon increase, and reduction of food intake, which are part of their non-glycemic effects[14,15].

The sex distribution of the sample revealed a slight male predominance (56.9%). Furthermore, most participants (85.6%) were Saudi nationals, representing a typical sample of the local population in the country, and 79.3% reported being married. This study revealed that women are more likely than men to be overweight or obese, consistent with a recent review that reported that women in Saudi Arabia are more likely to develop obesity in their middle and older years, which conforms to the study participants' average age[16]. Our results are consistent with earlier studies showing that married people may be more prone to weight gain for various reasons, including lifestyle changes and increased responsibilities[17,18].

Of the participants, 67.6% were employed. This indicates that a significant portion of the research population worked regularly, which may have influenced their overall well-being. Moreover, 28.1% of the participants had monthly incomes ranging from 10,000 to 19,999 SAR. As it falls between the middle and upper-middle classes, this income range suggests that a wide range of socioeconomic backgrounds were included in the study. Additionally, 42% reported having at least one chronic illness; 58% and 41.5% had diabetes and hypertension, respectively. These findings emphasize the importance of treating obesity as a risk factor for the onset of other chronic diseases as well as an isolated health issue, as numerous studies have shown a clear correlation between obesity and other chronic diseases[17,19].

Approximately, 26.1% of the participants were smokers; given that smoking is linked to many harmful health outcomes, such as an increased risk of cardiovascular disease and several types of cancer, its prevalence is a cause for concern. The correlation between smoking, obesity, and the risk of becoming overweight highlights the need for multifaceted therapies that address several risk factors simultaneously[20,21].

GLP-1 receptor agonists are thought to have an impact on weight loss by modulating hunger and satiety. In our study, semaglutide (Ozempic) (66%) and liraglutide (Saxenda) (39.4%) were the two most utilized GLP-1 agonists. Among these individuals, over half (54.8%) were taking the medications for both weight loss and diabetes. The average duration of the drug use was 12.43 ± 14.73 months, with 61% receiving the medication once a week via injection. Over the course of the treatment, the patients lost 10.11 ± 6.53 kg on average. In accordance with a prior study, the greatest mean changes in body weight with liraglutide 3.0 mg were -6.5 kg (95% CI: -7.4 to -5.5; $p < .001$) at 6 months of treatment[22]. Additionally, our findings align with previous studies that reported 4.4 kg of weight loss on average at 4 months, 7.0 kg at 4 months, and 6.4 kg at 3 months for participants in trials conducted in Switzerland, Canada, and Spain, respectively[23,24,25]. These findings suggest that the effects of GLP-1 receptor agonists on weight loss may differ, possibly due to the different homologies and administration frequencies of medications[26]. These findings are consistent with those of previous studies.

According to our results, most patients who used GLP-1 receptor agonists experienced gastrointestinal side effects, the most frequent being nausea (51.1%), anorexia (47.9%), and constipation (31.9%). Despite these adverse effects, patient adherence to the medication was unaffected, and for 55.3% of participants, the doctor decided to keep the patients on the same dosage despite the negative effects. The medications with the highest rates of adverse side effects were semaglutide (63.6%) and liraglutide (41.7%). Additionally, individuals without diabetes who were obese were more likely to experience adverse outcomes. According to an Indian study, stomach issues were the most frequently reported adverse effects. Exenatide and liraglutide were the two medications with the highest adverse effects[27]. According to another Saudi Arabian study, gastrointestinal antagonistic episodes rank among the most frequently reported side effects of semaglutide use[28].

Regarding managing side effects, a multidisciplinary consensus states that to prevent or at least lessen gastrointestinal side effects, patients must be educated about possible side effects and taught how to adhere to a set of rules. Healthcare professionals must understand that the best way to reduce the gastrointestinal side effects of GLP-1 receptor agonists is to provide thorough dietary education, flexibility during the dose escalation phase, and appropriate symptomatic treatment of persistent gastrointestinal adverse events[29]. Therefore, we encourage doctors to counsel patients about potential gastrointestinal side effects that may arise during treatment, refer them to gastroenterologists when necessary, and advise patients to follow up with dietitians regularly to develop a diet that is appropriate for them in the process of optimizing their health to maintain their quality of life and manage common adverse events.

The study findings provide insights into how patients perceive injectable treatment options. Several factors, such as dose frequency, convenience of use of the injection, and ease of administration, influence patient satisfaction.

Our findings align with previous research indicating that patients prefer medication profiles that involve single-use injections, once-weekly rather than once-daily dosing, which were linked to better glycemic control, less weight gain, and fewer adverse events in discrete choice experiments[30].

According to the current study, over one-third (30.9%) of participants reported being satisfied with their GLP-1 treatment, primarily due to diabetes control, weight loss, and minimal side effects from the medication. Furthermore, 78.7% of participants indicated how much they benefited from GLP-1, saying that they would recommend it to others. Other trials have reported a high patient satisfaction rate with GLP-1 medication, likely due to its association with decreased HbA1C levels and weight reduction[31, 32].

Using the Treatment Satisfaction Questionnaire for Medication (TSQM-9), which scores by domain and each domain scores range from 0 to 100, a previous cohort study discovered a high treatment satisfaction rate, with global satisfaction growing from 47.95 to 69.38 and efficacy climbing from baseline to 71.34. However, there was no difference in drug convenience between 69.06 and 71.34. As a result, they proposed that although liraglutide therapy increases treatment satisfaction and achieves a high adherence rate, once-daily subcutaneous injection of liraglutide did not improve convenience[32].

Hence, perceptions and preferences are important factors in adherence that affect the practical efficacy of GLP-1 receptor agonists.

LIMITATIONS

The limitations of our study include the fact that it was conducted at a single center in Jeddah, Saudi Arabia; therefore, it is difficult to generalize our findings to the entire Saudi Arabian population. As 685 patients of those who received the medication were excluded due to various reasons (non-response, refusing to share in the study, having a wrong phone number due to incorrect documentation; having a BMI < 27 kg/m^2 or aged < 18 years), future national multi-center studies in larger samples are encouraged to confirm the concluded results.

In addition, we could not access certain important laboratory results, such as HbA1C levels in patients with diabetes and lipid profiles, which may have added valuable information to our findings. Furthermore, the recurrent unavailability of the medications throughout the study period may have affected the efficacy of the treatment and the overall outcome of the study. Moreover, the use of a self-administered questionnaire may have introduced recall bias. Another limitation is that the study included participants with varying durations of GLP-1 agonist use, ranging from less than a month to over a year. This broad variability could significantly impact the interpretation of weight loss outcomes and adverse effects. Future studies should consider subgroup analyses based on the duration of medication use to offer more precise insights into the relationship between treatment duration and its effects. While this study recognizes that research on GLP-1 agonist use has been conducted worldwide, it is essential to note that this topic is still relatively new in Saudi Arabia. Although our findings may align with existing literature, examining GLP-1 agonist use in the Saudi population offers valuable insights that are currently absent from regional studies. This study serves as a foundational step toward understanding the implications and effectiveness of GLP-1 therapy within a local context. Future studies could build on these findings by investigating cultural and healthcare

factors specific to Saudi Arabia that may influence GLP-1 agonist use, enhancing the novelty and relevance of research in this emerging field.

CONCLUSION

According to this study, the two GLP-1 agonists most frequently prescribed are semaglutide and liraglutide, both of which have been shown to significantly reduce body weight during treatment. The most frequently reported side effects were nausea, anorexia, and gastrointestinal issues, particularly in non-diabetic individuals with obesity. Despite these issues, a sizable percentage of the participants reported being extremely happy with the results of their treatment and were willing to recommend GLP-1 therapy to others. Furthermore, future longitudinal studies with larger sample sizes and multi-center randomized controlled trials are required to generalize the effectiveness of GLP-1 agonists in the Saudi population. GLP-1 agonists should be used as adjuncts to lifestyle modifications. Given the limited long-term safety and efficacy data, cautious monitoring and further research in this area are required.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest that is related to this study and this article.

DISCLOSURE

The authors did not receive any form of commercial support, including compensation or financial assistance, for this case report. Additionally, the authors have no financial interest in any of the products, devices, or drugs mentioned in this article.

ETHICAL APPROVAL

Ethical approval was obtained from the Research Ethics Committee of King Abdulaziz University Medical Services Center, Jeddah, Saudi Arabia.

ACKNOWLEDGMENT

The authors gratefully acknowledge the cooperation of all participants.

REFERENCES CITED

[1] Almubark RA, Alqahtani S, Isnani AC, et al. Obesity in Saudi Arabia: RMHP [Internet]. Risk Management and Healthcare Policy. Risk Management and Healthcare Policy. 2022;15: 1179–1188

[2] Alluhidan M, Alsukait RF, Alghaith T, et al. Overweight and obesity in Saudi Arabia [Internet]. Open Knowledge Repository. Washington, DC: World Bank; 2022. Available from: https://openknowledge.worldbank.org/handle/10986/37723

[3] de Boer IH, Khunti K, Sadusky T, et al. Diabetes Management in Chronic Kidney Disease: A Consensus Report by the American Diabetes Association (ADA) and Kidney Disease: Improving Global Outcomes (KDIGO). Diabetes Care. 2022;45(12):3075-3090

[4] Mahapatra MK, Karuppasamy M, Sahoo BM. Semaglutide, a glucagon like peptide-1 receptor agonist with cardiovascular benefits for management of type 2 diabetes. Rev Endocr Metab Disord. 2022;23(3):521-539.

[5] Ard J, Fitch A, Fruh S, Herman L. Weight loss and maintenance related to the mechanism of action of glucagon-like peptide 1 receptor agonists [Internet]. Advances in therapy. U.S. National Library of Medicine; 2021 [cited 2023Jan21]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8189979/

[6] Barritt AS 4th, Marshman E, Noureddin M. Review article: role of glucagon-like peptide-1 receptor agonists in non-alcoholic steatohepatitis, obesity and diabetes-what hepatologists need to know. Aliment Pharmacol Ther. 2022;55(8):944-959.

[7] Chao AM, Tronieri JS, Amaro A, et al. Clinical Insight on Semaglutide for Chronic Weight Management in Adults: Patient Selection and Special Considerations. Drug Des Devel Ther. 2022;16:4449-4461.

[8] Taha MB, Yahya T, Satish P, et al. Glucagon-Like Peptide 1 Receptor Agonists: A Medication for Obesity Management. Curr Atheroscler Rep. 2022;24(8):643-654

[9] Salem V, AlHusseini N, Abdul Razack HI, et al. Prevalence, risk factors, and interventions for obesity in Saudi Arabia: A systematic review. Obes Rev. 2022;23(7):e13448.

[10] Collins L, Costello RA. Glucagon-Like Peptide-1 Receptor Agonists. [Updated 2024 Feb 29]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK551568/

[11] Filippatos TD, Panagiotopoulou TV, Elisaf MS. Adverse Effects of GLP-1 Receptor Agonists. Rev Diabet Stud. 2014;11(3-4):202-230.

[12] Onoviran OF, Li D, Toombs Smith S, et al. Effects of glucagon-like peptide 1 receptor agonists on comorbidities in older

patients with diabetes mellitus. Ther Adv Chronic Dis. 2019;10:2040622319862691.

[13] Friedrichsen M, Breitschaft A, Tadayon S, et al. The effect of semaglutide 2.4 mg once weekly on energy intake, appetite, control of eating, and gastric emptying in adults with obesity. Diabet Obes Metab. 2021;23:754–762.

[14] Baggio LL, Drucker DJ. Glucagon-like peptide-1 receptor co-agonists for treating metabolic disease. Mol Metab. 2021;46:101090.

[15] Paternoster S, Falasca M. Dissecting the physiology and pathophysiology of glucagon-like peptide-1. Front Endocrinol. 2018;2018:584-610.

[16] Wahabi H, Fayed AA, Shata Z, et al. The Impact of Age, Gender, Temporality, and Geographical Region on the Prevalence of Obesity and Overweight in Saudi Arabia: Scope of Evidence. Healthcare (Basel). 2023;11(8):1143-1166.

[17] Memish ZA, El Bcheraoui C, Tuffaha M, et al. Obesity and associated factors--Kingdom of Saudi Arabia, 2013. Prev Chronic Dis. 2014 9;11:E174.

[18] U.S. department of health and human services, Centers for Disease Control and Prevention, National Center for Health Statistics: Marital Status and Health: United States, 1999–2002. 2004. https://www.cdc.gov/nchs/data/ad/ad351.pdf

[19] Haslam DW, James WP. Obesity. Lancet. 2005;366(9492):1197-209.

[20] Dare S, Mackay DF, Pell JP. Relationship between smoking and obesity: a cross-sectional study of 499,504 middle-aged adults in the UK general population. PLoS One. 2015;10(4):e0123579.

[21] Powell-Wiley TM, Poirier P, Burke LE, et al. Obesity and Cardiovascular Disease: A Scientific Statement From the American Heart Association. Circulation. 2021;143(21):e984-e1010.

[22] Alshehri A, AlFaris N, Al Qahtani AM, et al. Clinical effectiveness of Liraglutide 3.0 mg and impact of weight loss in improving obesity-related comorbid conditions in King Fahad Medical City, Kingdom of Saudi Arabia: A real-world experience. Clin Obes. 2023;13(4):e12594.

[23] Haase CL, Serratore Achenbach MG, Lucrezi G, et al. Use of Liraglutide 3.0 mg for Weight Management in a Real-World Setting in Switzerland. Obes Facts. 2021;14(5):568-576.

[24] Wharton S, Liu A, Pakseresht A, et al. Real-World Clinical Effectiveness of Liraglutide 3.0 mg for Weight Management in Canada. Obesity (Silver Spring). 2019;27(6):917-924.

[25] Gorgojo-Martínez JJ, Basagoiti-Carreño B, Sanz-Velasco A, et al. Effectiveness and tolerability of orlistat and liraglutide in patients with obesity in a real-world setting: The XENSOR Study. Int J Clin Pract. 2019;73(11):e13399.

[26] Potts JE, Gray LJ, Brady EM, et al. The Effect of Glucagon-Like Peptide 1 Receptor Agonists on Weight Loss in Type 2 Diabetes: A Systematic Review and Mixed Treatment Comparison Meta-Analysis. PLoS One. 2015;10(6):e0126769..

[27] Shetty R, Basheer FT, Poojari PG, et al. Adverse drug reactions of GLP-1 agonists: A systematic review of case reports. Diabetes Metab Syndr. 2022Mar;16(3):102427.

[28] Alorfi NM, Algarni AS. Clinical Impact of Semaglutide, a Glucagon-Like Peptide-1 Receptor Agonist, on Obesity Management: A Review. Clin Pharmacol. 2022;14:61-67.

[29] Gorgojo-Martínez JJ, Mezquita-Raya P, Carretero-Gómez J, et al. Clinical Recommendations to Manage Gastrointestinal Adverse Events in Patients Treated with Glp-1 Receptor Agonists: A Multidisciplinary Expert Consensus. J Clin Med. 2022;12(1):145-162.

[30] Thieu VT, Robinson S, Kennedy-Martin T, Boye KS, Garcia-Perez LE. Patient preferences for glucagon-like peptide 1 receptor-agonist treatment attributes. Patient Prefer Adherence. 2019;13:561-576.

[31] Boye KS, Sapin H, Dong W, et al. Improved Glycaemic and Weight Management Are Associated with Better Quality of Life in People with Type 2 Diabetes Treated with Tirzepatide. Diabetes Ther. 2023;14(11):1867-1887.

[32] Zameer R, Kamin M, Raja U, et al. Effectiveness, Safety, and Patient Satisfaction of Liraglutide in Type 2 Diabetic Patients. Cureus. 2020;22;12(8):e9937.

ORIGINAL ARTICLE

The Association Between Playing Video Games and Thumb/Wrist Pain Among Medical Students at the King Abdulaziz University

Abdullah Altuwairqi, MD

Department of Orthopedic Surgery, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia

Correspondence
Dr. Abdullah Altuwairqi
Department of Orthopedic Surgery, Faculty of Medicine, King Abdulaziz University
PO Box 80205, Jeddah 21589
Kingdom of Saudi Arabia
e-M: abdullahabidt@hotmail.com

Submission: 19 Aug. 2024
Accepted: 22 Dec. 2024

Citation
Altuwairqi A. The association between playing video games and thumb/wrist pain among medical students at the King Abdulaziz University. JKAU Med Sci 2024; 31(2): 13-19. DOI: 10.4197/Med.31-2.2.

Abstract

De Quervain's disease is characterized by stennosing tenosynovitis in the first extensor wrist compartment. With the growing popularity of video gaming, the incidence of de Quervain's disease has continued to rise among teenagers and youths. There is a need to determine the correlation between gaming and de Quervain's disease. To assess the association between playing video games and thumb and wrist pain among medical students at King Abdulaziz University. This cross-sectional survey study was conducted among medical students at King Abdulaziz University between July 2020 and October 2020. Statistical analyses were performed using SPSS version 23. Most medical students (82%) played video games; among them, 36% and 59% reported using PlayStations and mobile phones, respectively. The mean ± SD pain severity was 1.63 ± 2.65, with 1.4% and 62.9% reported experiencing the most severe pain and no pain, respectively. Further, 12.9%, 15.1%, and 10.1% of the participants reported stretching their fingers before playing, having problems carrying things, and the pain affecting their daily activities, respectively. Most of our participants played video games mainly using mobile phones and PlayStations. However, a majority of them did not experience any pain; moreover, we observed an association of pain episodes and severity with the playing duration and devices used.

Keywords

De Quervain's disease, Video games, Thumb pain, Wrist pain, Association, Medical students

INTRODUCTION

Gaming has become an addiction in many young adults[1]. The World Health Organization (WHO) and the American Psychiatric Association (APA) have categorized gaming as a behavioral and mental health condition[2, 3]. Video games are defined as games played using an electronic system, including a digital phone, console, or computer. In Arab countries, video games are a popular leisure activity among adolescents and children[4]. Approximately 59%–73% of the youth have been shown to play video games on any given day[5, 6]. A study conducted in Abha among secondary school students reported a high prevalence of playing video games, with 80% and 75% of the students reporting that they played video games and owned a video game machine, respectively[4].

Gaming is associated with several diseases and complications. An Indian study on medical students reported an increase in gaming disorders, which led to anxiety, psychological disturbance, sleep, and mood disorders, and physical complaints such as headaches.[7] Another Saudi Arabian study reported an association of gaming addiction with stress among adolescents[1].

De Quervain's disease is characterized by stenosing tenosynovitis of the first dorsal wrist compartment. It is characterized by pain with gradual onset, which may be exacerbated by thumb abduction, grasping, and ulnar deviation of the wrist[8].[4] The etiology of De Quervain's disease is thought to be sustained or repetitive tension on the tendons of the first dorsal compartment. This tension causes a fibroblastic response, which in turn leads to swelling and thickening of the compartment. Moreover, an individual experiences discomfort while using the wrist and hand[8].

De Quervain's disease is the most common inflammatory wrist tendon lesion. Inflammatory wrist and hand tendon lesions have become increasingly common with the evolution of electronic systems[9]. A study conducted in Jeddah, Saudi Arabia, which included 338 university students reported a positive correlation of smartphone use with De Quervain's disease among medical and non-medical students[10]. This study aimed to assess the association of thumb and wrist pain with playing video games among medical students.

METHODS

PARTICIPANTS AND STUDY DESIGN

This cross-sectional survey study was conducted among medical students at King Abdulaziz University between July 2020 and October 2020.

We randomly included 140 medical students. This study was approved by the ethical committee of King Abdulaziz University (reference number 164-21).

THE STUDY TOOL AND SCORING

The survey was sent to the students. The survey involved a two-step process. First, the students received a link to a video explaining how to perform Finkelstein's test. Moreover, information regarding its purpose was attached to the questionnaire to allow participants to perform the test on their own before answering the follow-up questions. The presence of pain during the test was considered a positive result.

Regardless of Finkelstein's test results, the participants answered a series of closed-ended questions to assess the frequency and duration of playing video games across different consoles (PlayStation, Xbox, Nintendo, PC, and smartphones), as well as whether there were pain episodes during or after playing.

The severity of pain was assessed on a scale of 0–10, where 10 reflected the most severe pain, with accompanying interference of daily activities, pain with moving objects, and episodes of nighttime awakening due to pain. The pain duration was categorized as < 10 minutes, 10–60 minutes, > 1 hour, or constant pain throughout the day. Pain-related experiences, including weakness, numbness, and improvement or worsening with finger movement, were also assessed.

Moreover, precautionary measures, including taking breaks while playing video games and stretching frequency, were assessed. Additionally, a well-established risk factor, which is the duration of playing, is also evaluated.

STATISTICAL ANALYSIS

Data were collected and analyzed using SPSS version 23 (IBM SPSS, IBM Corp., Armonk, NY, USA). Data are expressed as numbers (%). Between-group comparisons were performed using the chi-square test. Statistical significance was set at $P < 0.05$.

RESULTS

Most participants (82.0%) played video games. Regarding the daily duration of playing video games, most participants reported 1–3 hours (31.7%), followed by > 5 hours (18.7%), > 3–5 hours (15.8%), < 1 hours (12.9%), and depending on the free time (2.9%). Most participants played video games daily (36.7%), followed by 1–2 times a week (28.1%), 2–4 times a week (13.7%), and monthly (3.6%). Moreover, 12.9% and 15.1% stretched their fingers before playing and had problems with carrying things, respectively. Regarding taking breaks when playing, most participants answered sometimes (46.8%), followed by no (29.5%) and yes (23.7%), with significant differences among them ($P = 0.003$). Most participants did not experience pain episodes (70.5%), while 18.0%, 7.9%, 2.2%, and 1.4% had pain episodes for < 10 minutes, 10–60 minutes, > 60 minutes, and throughout the day, respectively, with significant differences among them ($P < 0.0001$). Pain affected daily activities in 89.9% of the participants, with 7.9% seeing doctors for this problem. Regarding nighttime awakenings induced by wrist/thumb pain, 11.5%, 3.6%, 2.2%, and 82.7% of the participants reported that they rarely, usually, always, and never woke up, respectively. Wrist pain was associated with finger numbness and hand weakness in 15.8% of the participants; moreover, it worsened and improved with finger movement in 12.2% of the participants. Further, 62.6%, 16.5%, 11.5%, and 9.4% of the participants reported no pain, pain during activities, constant pain, and episodic pain, respectively. The mean pain score was 1.63. Regarding the console used for video games, most participants used mobile (59.0%), followed by PlayStation (36.0%), PC (33.1%), Nintendo (18.7%), and Xbox (10.1%), with significant differences among them ($P < 0.0001$) (Table 1).

Mild, mild-to-moderate, moderate, moderate-to-severe, and severe pain were mostly reported by participants who played > 3–5 hours/day (40.0%), > 5 hours/day (37.5%), > 5 hours/day (66.7%), 1–3 hours/day (50.0%), and no playing (50.0%) (Table 2).

Table 1. The association between playing video games and thumb/wrist pain: A cross-sectional study

Questions	Value	*P*- value
Do you play video games?		0.0001
Yes	114 (82.0%)	
No	25 (18.0%)	
For how long do you play video games per day		0.0001
None	25 (18.0%)	
Less than an hour	18 (12.9%)	
1–3 hours	44 (31.7%)	
3–5 hours	22 (15.8%)	
> 5 hours	26 (18.7%)	
Others (depending on the free time)	4 (2.9%)	
How often do you play video games?		0.0001
Daily	51 (36.7%)	
1–2 times a week	39 (28.1%)	
2–4 times a week	19 (13.7%)	
Monthly	5 (3.6%)	
Never	25 (18.0%)	

Questions	Value	*P*- value
Do you stretch your fingers before you play?		0.0001
Yes	18 (12.9%)	
No	121 (87.1%)	
Do you have problems carrying things?		0.0001
Yes	21 (15.1%)	
No	118 (84.9%)	
Do you take rest breaks when playing?		0.003
Yes	33 (23.7%)	
No	41 (29.5%)	
Sometimes	65 (46.8%)	
How long, on average, does a pain episode last during the daytime (minutes)?		0.0001
< 10 min	25 (18.0%)	
10–60 min	11 (7.9%)	
> 60 min	3 (2.2%)	
Constant	2 (1.4%)	

Table 1. **The association between playing video games and thumb/wrist pain: A cross-sectional study.–Continued**

Questions	Value	*P*- value
Has this problem affected your daily activities?		0.0001
Yes	14 (10.1%)	
No	125 (89.9%)	
Have you ever seen a doctor for this problem?		
Yes	11 (7.9%)	
No	128 (92.1%)	
How often does your wrist/thumb pain wake you up?		0.0001
Always	3 (2.2%)	
Usually	5 (3.6%)	
Rarely	16 (11.5%)	
Never	115 (82.7%)	
What is associated with your wrist pain?		0.0001
Finger numbness	22 (15.8%)	
Worse with finger movement	17 (12.2%)	
Better with finger movement	17 (12.2%)	
Hand weakness	22 (15.8%)	
Describe the timing of your pain?		0.0001
Episodically	13 (9.4%)	
Constant	16 (11.5%)	
During activity	23 (16.5%)	
No pain	87 (62.6%)	
What is the pain score out of 10?		
Mean+/-SD	1.63 ± 2.65 (0.0-10.0)	
0	87 (62.9%)	
1	9 (6.5%)	
2	6 (4.3%)	
3	7 (5.0%)	
4	9 (6.5%)	
5	2 (1.4%)	
6	7 (5.0%)	
7	5 (3.6%)	
8	3 (2.2%)	
9	2 (1.4%)	
What console do you use for video games?		
PlayStation	89 (36.0%)	0.001
Xbox	14 (10.1%)	0.0001
PC	46 (33.1%)	0.0001
Nintendo	26 (18.7%)	0.0001
Mobile phone	82 (59.0%)	0.034

Table 2. **Cross table between pain severity and length of playing video games per day**

Pain Severity (pain score)	How long do you play video games per day?					
	None	< 1 hour	1–3 hours	3–5 hours	> 5 hours	Others
No pain (0) (n = 87)	19 (21.8%)	12 (13.8%)	26 (29.9%)	11 (12.6%)	17 (19.5%)	2 (2.3%)
Mild pain (1–2) (n = 15)	1 (6.7%)	2 (13.3%)	4 (26.7%)	6 (40.0%)	2 (13.3%)	–
Mild to Moderate pain (3–4) (n = 16)	–	3 (18.8%)	4 (25.0%)	3 (18.8%)	6 (37.5%)	–
Moderate (5–6) (n = 9)	2 (22.2%)	–	6 (66.7%)	–	1 (11.1%)	–
Moderate to severe (7–8) (n = 8)	1 (12.5.0%)	1 (12.5%)	4 (50.0%)	1 (12.5%)	–	1 (12.5%)
Severe (9–10) (n = 4)	2 (50.0%)	–	–	1 (25.0%)	–	1 (25.0%)

Data are expressed as numbers (%).

Mild, mild-to-moderate, and moderate pain were mostly reported by participants who played daily (46.7%, 43.8%, and 55.6%, respectively). Moderate-to-severe and severe pain was mostly reported by participants who played > 2–4 times per week (50.0%) and never played (50.0%), respectively (Table 3).

Pain episodes lasting < 10 min, 10–60 min, and > 60 min were mostly observed in participants who played 1–3 h/day (28.0%, 36.4%, and 66.7%, respectively). Constant pain was mostly reported in participants who played < 1 h and 1–3 h/day (50.0% for both), while no pain episodes were reported by participants who played 1–3 hours (30.6%) (Table 4).

Mild pain was mostly reported in participants with constant pain (53.3%). Mild-to-moderate and moderate pain were mostly reported during activity (62.5% and 55.6%, respectively). Moderate-to-severe pain was mostly reported in participants with episodic and constant pain (37.5% for both), while severe pain was mostly reported in participants with constant pain (50.0%) (Table 5).

Table 3. Cross table between pain severity and how many days they play video games per week

Pain Severity (pain score)	How often do you play video games per week?				
	Daily	1–2 times per week	2–4 times per week	Monthly	Never
No pain (0) (n = 87)	31 (35.6%)	27 (31.0%)	8 (9.2%)	3 (3.4%)	18 (20.7%)
Mild pain (1–2) (n = 15)	7 (46.7%)	3 (20.0%)	4 (26.7%)	–	1 (6.7%)
Mild to Moderate pain (3–4) (n = 16)	7 (43.8%)	6 (37.5%)	2 (2.5%) 2	1 (6.2%)	–
Moderate (5–6) (n = 9)	5 (55.6%)	1 (11.1%)	1 (11.1%)	–	2 (22.2%)
Moderate to severe (7–8) (n = 8)	–	2 (25.0%)	4 (50.0%)	1 (25.0%)	1 (25.0%)
Severe (9–10) (n = 4)	1 (25.0%)	1 (25.0%)	–	–	2 (50.0%)

Data are expressed as numbers (%).

Table 4. Cross table between the duration of the pain episode and the duration of playing video games per day

How long do the pain episodes last?	How long do you play video games per day?					
	None	< 1 hour	1–3 hours	3–5 hours	> 5 hours	Others
< 10 min (n = 25)	2 (8.0%)	4 (16.0%)	7 (28.0%)	5 (20.0%)	6 (24.0%)	1 (4.0%)
10–60 min (n = 11)	2 (18.2%)	–	4 (36.4%)	3 (27.3%)	2 (18.2%)	–
> 60 min (n = 3)	1(33.3%)	–	2 (66.7%)	–	–	–
Constant (n = 2)	–	1 (50.0%)	1 (50.0%)	–	–	–
Never had episode (n = 98)	21 (21.4%)	13 (13.3%)	30 (30.6%)	14 (14.3%)	18 (18.4%)	2 (2.0%)

Data are expressed as numbers (%).

Table 5. Cross table between pain severity and timing of pain

Pain Severity (pain score)	Timing of Pain			
	Episodically	Constant	During activity	No pain
No pain (0) (n = 87	–	–	–	(100.0%) 87
Mild pain (1–2) (n = 15	2 (13.3%)	8 (53.3%)	5 (33.3%)	–
Mild-to-moderate pain (3–4) (n = 16)	3 (18.8%)	3 (18.8%)	0 (62.5%)	–
Moderate (5–6) (n = 9)	4 (44.4%)	–	5 (55.6%)	–
Moderate to severe (7–8) (n = 8)	3 (37.5%)	3 (37.5%)	2 (25.0%)	–
Severe (9–10) (n = 4)	1 (25.0%)	2 (50.0%)	1 (25.0%)	–

Data are expressed as numbers (%).

Table 6. Cross table between pain severity and console used

Pain Severity (pain score)	Console Used for Video Games				
	PlayStation	Xbox	PC	Nintendo	Mobile phone
No pain (0) (n = 87)	51 (58.6%)	8 (6.9%)	30 (34.5%)	16 (18.4%)	44 (50.6%)
Mild pain (1–2) (n = 15)	13 (86.7%)	2 (13.3%)	6 (40.0%)	2 (13.3%)	13 (86.7%)
Mild-to-moderate pain (3–4) (n = 16)	13 (81.2%)	3 (18.8%)	9 (62.5%)	4 (25.0%)	15 (93.8%)
Moderate (5–6) (n = 9)	6 (66.7%)	–	–	3 (33.3%)	6 (66.7%)
Moderate-to-severe (7–8) (n = 8)	4 (50.0%)	2 (25.0%)	1 (12.5%)	1 (12.5%)	3 (37.5%)
Severe (9–10) (n = 4)	2 (50.0%)	1 (25.0%)	–	–	1 (25.0%)

Data are expressed as numbers (%).

No pain was mostly reported by participants with a PlayStation (58.6%). Mild pain was mostly reported in participants using a PlayStation and mobile phone (86.7% for both). Mild-to-moderate pain was mostly reported in participants using a mobile phone (93.8%). Moderate was mostly reported in participants using a PlayStation and mobile phone (66.7% for both). Moderate-to-severe and severe pain was mostly reported in participants using a PlayStation (50.0% for both) (Table 6).

DISCUSSION

Our findings revealed a high prevalence of playing video games among medical students, with 82% of our participants reporting playing video games. Most students reported playing video games for 1–3 hours/daily. The most frequently used device for playing games was the mobile phone (59%), followed by the PlayStation (36%) and personal computer (33.1%).

A study conducted in Jeddah that enrolled 387 medical students showed that 66.4% of students had smartphone addiction[11], which was higher than that in our study. However, the previous study did not report whether the students used smartphones for gaming or other purposes.

We investigated the pain and its effect on students, with 2.2% and 82.7% of the students reporting that they always and never woke up, respectively, due to wrist and thumb pain. The mean score of pain severity was only 1.63; moreover, 62.9% and 1.4% reported no pain and the most severe pain, respectively. Regarding the pain impact, most patients denied any pain impact on their daily activities and did not visit a doctor for pain. The most common consequences of pain were finger numbness and weakness. Additionally, only a few students reported stretching their fingers before playing and having problems carrying things. Furthermore, we investigated factors that may affect pain severity.

In addition, we analyzed the association between the pattern of playing video games and pain severity. Playing for 1–3 and > 5 hours per day was associated with moderate-to-severe and mild-to-moderate pain, respectively. Notably, two students who reported no playing showed severe pain. Daily playing was associated with mild-to-moderate pain. Additionally, daily playing for 1–3 hours was significantly associated with pain episodes lasting from < 10 minutes to > 60 minutes. Further, the severity of pain was associated with no finger stretching before playing. Mobile phones and PlayStations were associated with the severity of pain.

A study reported that overusing the thumb for mobile texting is a risk factor for de Quervain's disease[12]. It implies that using mobile phones for a long duration could be associated with the development of de Quervain's disease. This is consistent with our findings that using mobile phones and PlayStations to play games for long durations was associated with pain development, duration, and severity. In our study, a small proportion of students reported experiencing pain, which could be attributed to a short duration spent playing video games or playing only during vacation. Further research is required to better understand the low pain prevalence among students playing video games.

A cross-sectional study of 833 adolescents reported similar findings. Specifically, high usage of computers (99%) and video games (58%) was found; however, the reported pain prevalence was only 39.4%. Additionally,

it should be noted that this previous study investigated musculoskeletal, rather than thumb or wrist pain[13].

A previous study that included 500 students reported that the incidence of de Quervain's disease was 49% among students who used mobile phones to play video games. Moreover, the risk of disease increased with a longer duration of playing, frequent playing, and changes in wrist position[9].

A study conducted in Jeddah reported that de Quervain's disease had a prevalence rate of 68.9% among 338 students. Moreover, it reported a positive correlation between the use of phones for gaming and de Quervain's disease[10]. Another study conducted on medical students in Jeddah reported a significant association between heavy smartphone use and thumb/wrist pain[11].

CONCLUSION

Our findings revealed a high prevalence of playing video games among medical students, with mobile phones and PlayStations as the most common devices. There was a very low mean score of pain severity since most of the patients reported no pain. Pain severity was associated with playing daily for a long duration using mobile phones and PlayStations; moreover, the duration of pain episodes was associated with the playing duration.

ACKNOWLEDGMENT

The author thanks King Abdulaziz University for their support in this research. I would also like to thank Abdulelah K. Bahabri for his active contribution to the data collection process..

CONFLICT OF INTEREST

The author declared that there is no conflict of interest that is related to this study and this article.

DISCLOSURE

The author did not receive any form of commercial support, including compensation or financial assistance, for this case report. Additionally, the author has no financial interest in any of the products, devices, or drugs mentioned in this article.

REFERENCES CITED

[1] Rajab AM, Zaghloul MS, Enabi S, Rajab TM, Al-Khani AM, Basalah A, et al. Gaming addiction and perceived stress among Saudi adolescents. Addict Behav Rep. 2020; 11: 100261.

[2] American Psychiatric Association. Conditions for further study Diagnostic and Statistical Manual of Mental Disorders. 5th ed. Arlington, VA: American Psychiatric Association; 2013.

[3] World Health Organization. International statistical classification of diseases and related health problems, 11th revision (ICD-11); 2019. Available from: http://id.who.int/icd/entity/ 1448597234.

[4] Awadalla N, Hadram M, Alshahrani A, Hadram Y. Association of video gaming with some risky behaviors of secondary school adolescents in Abha, Southwestern Saudi Arabia. J Egypt Public Health Assoc. 2017; 92: 18-28.

[5] Desai RA, Krishnan-Sarin S, Cavallo D, Potenza MN. Video-gaming among high school students: health correlates, gender differences, and problematic gaming. Pediatrics. 2010; 126: e1414-e1424.

[6] Roberts D, Foehr U, Rideout V. Generation M: media in the lives of 8–18 year-olds. Menlo Park, CA: Henry J Kaiser Foundation; 2005.

[7] Yarasani P, Shaik RS, Myla AR. Prevalence of addiction to online video games: Gaming disorder among medical students. Int J Community Med Public Health. 2018; 5: 4237-4241.

[8] Ilyas AM, Ast M, Schaffer AA, Thoder J. De quervain tenosynovitis of the wrist. J Am Acad Orthop Surg. 2007; 15: 757-764.

[9] Ma T, Song L, Ning S, Wang H, Zhang G, Wu Z. Relationship between the incidence of de Quervain's disease among teenagers and mobile gaming. Int Orthop. 2019; 43: 2587-2592.

[10] Reada B, Alshaebi N, Almaghrabi K, Alshuaibi A, Abulnaja A, Alzahrani K. Prevalence and awareness evaluation of De quervain's tenosynovitis among students in the Kingdom of Saudi Arabia. Int J Pharm Res Allied Sci. 2020; 9: 151-157.

[11] Baabdullah A, Bokhary D, Kabli Y, Saggaf O, Daiwali M, Hamdi A. The association between smartphone addiction and thumb/wrist pain: A cross-sectional study. Medicine. 2020.

[12] Ali M, Asim M, Danish SH, Ahmad F, Iqbal A, Hasan SD. Frequency of De Quervain's tenosynovitis and its association with SMS texting. Muscles Ligaments Tendons J. 2014; 4: 74.

[13] Zapata AL, Moraes AJ, Leone C, Doria-Filho U, Silva CA. Pain and musculoskeletal pain syndromes related to computer and video game use in adolescents. Eur J Pediatr. 2006; 165: 408-414.

ORIGINAL ARTICLE

Recombination Dynamics Divergent of the RGD Region of Penton, Hexon, and Fibre Genes for Adenovirus

Basem A. Jawa[1], Khulud A. Alhazmi[2], Rehab G. Alqurashi[3], Ghazi S. Alkhaldi[4], Karem A. Ibrahem[5], Rashad G. Rawzi[1], Fawzi S. Almatrafi[1], Mohammed A. Moazen[1], Mohammed A. Kurdi[4], Fouad A. Bantn[1], Ahmed A. Khan[4], Fahad T. Faqeeh[1], Ahmad A. Saati[4], Eman A. Mulla[4], Edhah S. Alsaeedi[4], Zainab A. Alkhayri[4], Zain Z. Alzubaidi[4], Mazin S. Alqurashi[1], Thamer N. Alotaibi[1], Alaa M. Alsaggaf[6], Mohamed A. Bazaid[4], Faisal H. Alzahrani[1], Shirin M. Kaki[1], Abeer H. Najjar[7], Ahmad M Sait[9,10] and Hammad H. Alsuwat[8]

[1]Department of Laboratory and Blood Bank, Alnoor Specialist Hospital, Makkah Health Cluster, Makkah, Saudi Arabia
[2]Department of Microbiology and Parasitology, Faculty of Medicine, Umm Alqura University, Makkah, Saudi Arabia
[3]Infection prevention and control, Makkah medical cluster, Ministry of Health, Makkah, Saudi Arabia
[4]Depratment of Laboratory, Regional Lab, Ministry of Health, Makkah, Saudi Arabia.
[5]Department of Clinical Microbiology and Immunology, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia
[6]Department of Laboratory and Blood Bank, King Salman Bin Abdulaziz Medical City, Madinah Health Cluster, Madinah, Saudi Arabia
[7]Department of Poison Control Forensic Chemistry, Ministry of Health, Makkah, Saudi Arabia
[8]Department of Laboratory and Blood Bank, Asir Health Cluster, Asir, Saudi Arabia
[9]Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia
[10]Regenerative Medicine Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah 21589, Saudi Arabia

Correspondence
Dr. Basem A. Jawa
Department of Laboratory and Blood Bank, Alnoor Specialist Hospital, Ministry of Health, Makkah 21955, Saudi Arabia
e-M: bajawa@moh.gov.sa

Submission: 26 Oct. 2024
Accepted: 15 Nov. 2024

Citation
Jawa BA, Alhazmi KA, Alqurashi RG, Alkhaldi GS, Ibrahem KA, Rawzi RG, Almatrafi FS, Moazen MA, Kurdi MA, Bantn FA, Khan AA, Faqeeh FT, Saati AA, Mulla EA, Alsaeedi ES, Alkhayri ZA, Alzubaidi ZZ, Alqurashi MS, Alotaibi TN, Alsaggaf AM, Bazaid MA, Alzahrani FH, Kaki SM, Najjar AH. Recombination dynamics divergent of the RGD region of penton, hexon, and fibre genes for adenovirus. JKAU Med Sci 2024; 31(2): 21-34. DOI: 10.4197/Med.31-2.3.

Abstract

A Recent research on adenoviruses has mainly concentrated on their potential as vectors for gene therapy and vaccines, with less attention given to their evolutionary patterns and genome stability. Traditional typing methods, which rely on serological assays, have proven inadequate for classifying adenoviruses due to their genetic diversity. To address this, sequencing is often limited to key regions such as the hexon and fiber genes. However, recent studies suggest that relying solely on these regions may not fully capture the genetic landscape of adenoviruses. Notably, multiple recombination events have been identified within the adenovirus genome, raising concerns about the reliability of partial sequencing approaches for precise typing. In this study, we sequenced the penton gene in nine adenovirus isolates with previously characterized hexon and fiber types. Our analysis revealed that six of these isolates had penton sequences distinct from their assigned hexon and fiber types, suggesting recombination between adenovirus types. Additionally, four of these isolates displayed penton gene sequences that diverged significantly from known adenovirus types. Further examination uncovered potential recombination within the penton gene itself, particularly between the hypervariable region and the RGD (Arg-Gly-Asp) loop. These findings underscore the high prevalence of recombination in species D adenoviruses, not only between the hexon, fiber, and penton genes but also within the penton gene itself. This points to the limitations of hexon/fiber typing alone and emphasizes the need for full-genome sequencing to ensure accurate adenovirus classification. These insights are critical for advancing adenovirus-based vector development, as they contribute to a better understanding of adenovirus genetic stability and evolution.

Keywords

Adenovirus genome recombination, Adenovirus vector development, Full-genome sequencing, Evolutionary diversity

INTRODUCTION

Adenoviruses (AdVs) are double-stranded DNA viruses belonging to the family Adenoviridae, which encompass a wide range of subtypes infecting various species, including humans, animals, and birds[1]. Human adenoviruses (HAdVs) are significant pathogens responsible for a variety of clinical manifestations, including respiratory infections, gastroenteritis, conjunctivitis, and in some cases, severe disease in immunocompromised individuals[2]. The substantial genomic diversity among HAdVs necessitates precise and robust methods for classification and typing, which are crucial for epidemiological surveillance, clinical diagnostics, and the development of targeted therapeutic interventions[3].

Historically, adenovirus classification has relied on serological typing based on neutralization assays[4]. However, these methods are time-consuming, labor-intensive, and sometimes need more sensitivity and specificity[5]. The advent of molecular techniques, particularly polymerase chain reaction (PCR) and sequencing, has revolutionized adenovirus typing, providing more precise and rapid identification of HAdV genotypes[6]. Among the various genomic regions analyzed, the hypervariable region (HVR) of the penton gene has emerged as a particularly promising target for molecular typing due to its high sequence variability[7]. The penton base protein, a crucial structural component of the adenovirus capsid, plays a pivotal role in virus-host interactions, including cell entry and immune evasion[8]. The HVR within the penton gene exhibits significant sequence diversity, reflecting the evolutionary pressures exerted by host immune responses and contributing to the genetic heterogeneity observed among adenovirus subtypes[8].

Consequently, the HVR region serves as a valuable marker for distinguishing between closely related adenovirus strains and for identifying novel genotypes[9]. The significance of this research extends beyond mere classification. Accurate typing of adenoviruses is essential for understanding the epidemiology of HAdV infections, particularly in outbreak settings where rapid identification of the causative agent can inform public health responses[10]. Furthermore, the identification of novel variants and recombination events can provide insights into adenovirus evolution and pathogenesis, with implications for vaccine design and antiviral strategies[11].

The penton base protein is a crucial component of the adenovirus capsid, forming the penton complex along with the fiber protein. It plays a pivotal role in virus attachment and entry into host cells, interacting with integrins to mediate internalization[12]. The penton base gene exhibits significant genetic variability, particularly in its hypervariable regions (HVRs), which are subject to immune selection pressures. The HVRs within the penton base gene are characterized by high sequence diversity, making them ideal targets for molecular typing. This variability reflects the evolutionary pressures exerted by host immune responses and contributes to the antigenic diversity of HAdVs[13]. Studies have shown that the penton HVRs can distinguish between closely related adenovirus types and identify novel variants[14,15].

Adenovirus classification has been based on serological methods, particularly neutralization assays, which identify virus types based on their reactivity with specific antisera[16]. While serological typing has been instrumental in the initial classification of AdVs, it has several limitations, including the requirement for virus culture, cross-reactivity between closely related types, and limited sensitivity. The advent of molecular techniques has revolutionized adenovirus typing. Polymerase chain reaction (PCR) and sequencing have emerged as the gold standards for accurate and rapid identification of HAdV genotypes[17]. Molecular typing targets various genomic regions such as the hexon, fiber, and penton genes, which encode key structural proteins of the virus capsid.

The objective of this study is to investigate the utility of limited typing of the hypervariable region (HVR) of the penton gene in adenovirus for identifying virus genotypes. Specifically, the study aims to assess whether sequencing and phylogenetic analysis of the penton HVR region are sufficient to classify subtypes of human adenovirus (HAdV). This involves examining the nucleotide and amino acid sequences of the penton HVR region from clinical isolates and comparing them with known reference sequences to determine their relatedness and potential novelty. The study also aims to evaluate the divergence between isolates and known types, assess potential recombination events within the penton gene, and discuss the implications of these findings for adenovirus typing and vaccine development.

METHODOLOGY

DNA EXTRACTION

The extraction of DNA was conducted using the QIAamp (QIAGEN, a biotechnology company based in Germany) DNA Mini Kit, in accordance with the manufacturer's instructions. Initially, Q-Pro and AL buffers were added to the sample contained within a microcentrifuge tube. The mixture was vortexed thoroughly and subsequently incubated at 56°C for 15 minutes to facilitate cell lysis. After incubation, the tube was centrifuged and ethanol was added to the mixture to precipitate the DNA. The resulting solution was then transferred to a QIAamp spin column and centrifuged for 1 minute to allow the DNA to bind to the silica membrane within the column. The filtrate was discarded, and the column was placed into a new collection tube. To wash the DNA, AW1 buffer was added to the column, which was then centrifuged for 1 minute. This step was followed by another wash with AW2 buffer, after which the column was centrifuged for 3 minutes to ensure the thorough removal of contaminants. The final elution of the purified DNA was performed by adding AE buffer to the column and centrifuging for 1 minute. The eluted DNA was then stored at -20°C for subsequent use.

POLYMERASE CHAIN REACTION (PCR)

Conventional PCR was employed to amplify the partial penton region, encompassing the hypervariable region (HVR). The specific primers used for amplification were designed as follows: the forward primer sequence was TTCGCAAGAAGCAACCTTT, and the reverse primer sequence was TCTTGCATGAGGTCCGG. The PCR master mix (Thermo Fisher Scientific, United States) was prepared with the following components: 5 µl of 10X PCR buffer, 1 µl of 0.2 mM dNTPs, 0.5 µl of 0.2 µM forward primer, 0.5 µl of 0.2 µM reverse primer, 0.25 µl of 1.25 units/µl Taq DNA polymerase, and 37.75 µl of sterile distilled water. To this mixture, 5 µl of the DNA template or a control sample was added, making the final reaction volume 50 µl.

The PCR cycling program began with an initial denaturation step at 95°C for 15 minutes to activate the Taq DNA polymerase. This was followed by 40 cycles of amplification, each consisting of 20 seconds at 94°C for denaturation, 20 seconds at 57°C for primer annealing, and 40 seconds at 72°C for elongation. The final extension step involved a 5-minute incubation at 72°C to ensure complete extension of the amplified products. After PCR amplification, the amplicons were analyzed by electrophoresis on a 2% agarose E-gel. The molecular weights of the PCR products were determined by comparison with a DNA ladder, which contained fragments ranging from 100 bp to 2500 bp.

forward primer	TTCGCAAGAAGCAACCTTT,
reverse primer	TCTTGCATGAGGTCCGG

PHYLOGENETIC ANALYSIS

The nucleotide sequences of the penton region, specifically the 918 bases corresponding to the HVR of HAdV-D9, along with the deduced amino acid sequences, were used to construct phylogenetic trees to elucidate the evolutionary relationships among species D human adenoviruses. Sequence editing was performed using BioEdit version 7.0.5. Multiple sequence alignments were calculated using ClustalX version 1.83 to ensure precise alignment of the sequences.

Phylogenetic trees were constructed utilizing the Phylo-Win software version 2. To assess the robustness of the phylogenetic groupings, bootstrap analysis was performed with 1,000 pseudoreplicates. The phylogenetic trees were subsequently visualized and drawn using TreeView version 1.6.6. This comprehensive analysis allowed for the detailed examination of the genetic relationships and evolutionary patterns among the adenovirus isolates under study, particularly within the hypervariable regions of the penton gene.

RESULTS

PCR AMPLIFICATION OF CLINICAL ISOLATE

The DNA from the nine adenovirus isolates was extracted, and a specific region of the penton gene was amplified using PCR. Two primers were designed to generate around 600-base pair fragments of the penton gene. The amplified products, along with positive control fragments of 400 base pairs, were visualized on a gel electrophoresis image (Figure 1).

TYPING AND PHYLOGENETIC ANALYSIS OF ADENOVIRUS ISOLATES

Nine clinical samples from individuals with AIDS were analyzed using DNA sequencing and the phylogenetic analysis of the hexon and fiber genes. These isolates were identified as belonging to the HAdV-D species,

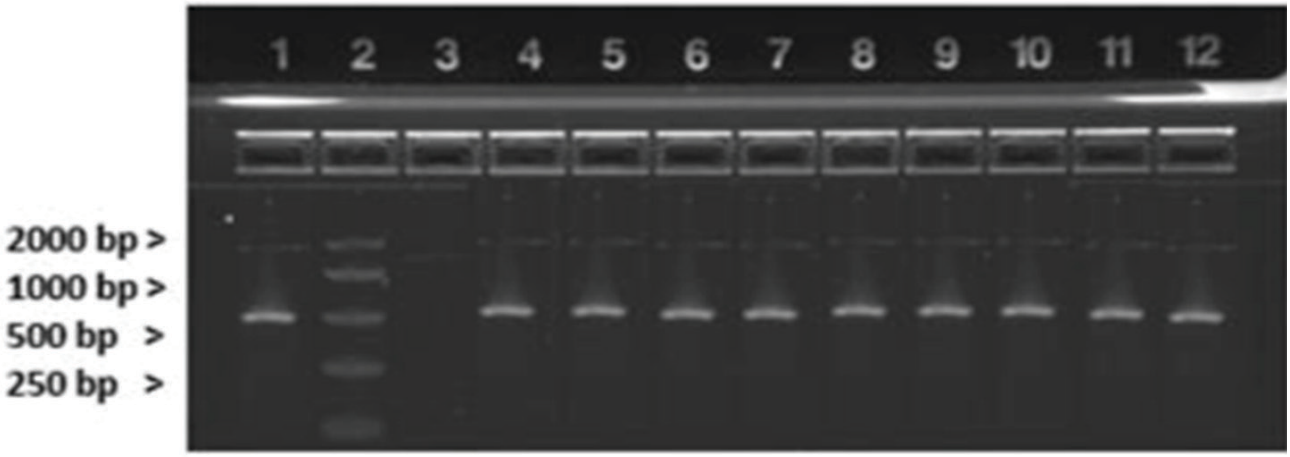


Figure 1. **Two percent E-agarose gel of RGD region. The size of the region is 551 base pairs. Lane 1: Positive Control. Lane 2: Quantitative DNA easy ladder. Lane 3: Negative Control. Lane 4: Sample 1. Lane 5: Sample 2. Lane 6: Sample 3. Lane 7: Sample 4. Lane 8: Sample 5. Lane 9: sample 6. Lane 10: Sample 7. Lane 11: Sample 8. Lane 12: Sample 9.**

Table 1. **The HAdV-D types of samples for Hexon and Fibre genes**

Sample No	HAdV-D Type
1	51
2	49
3	9
4	9
5	23
6	47
7	47
8	43
9	26

with consistent typing results for both the hexon and fiber regions. The previous typing results are summarized in Table 1.

PHYLOGENETIC ANALYSIS REVEALS GENETIC RELATIONSHIPS OF NEW ADENOVIRUS TYPES WITH CLINICAL ISOLATES

The phylogenetic analysis of the penton gene revealed the presence of multiple distinct clades within the HAdV-D species. Notably, several clinical isolates from AIDS patients clustered closely with specific reference strains, indicating their classification within established adenovirus types. However, the tree also highlighted the presence of several isolates that did not cluster with any known HAdV type, suggesting potential novel or recombinant strains. These findings underscore the genetic diversity within the HAdV-D species and emphasize the need for comprehensive genomic analysis to accurately characterize and classify adenoviruses, particularly in immunocompromised individuals, as shown in Figures 2 and 3.

The phylogenetic analysis of the penton gene revealed a complex pattern of divergence within the HAdV-D species. While some clinical isolates clustered closely with established reference strains, indicating a high degree of sequence similarity, others exhibited substantial divergence. This divergence may be attributed to several factors, including genetic recombination, rapid evolution, and potential adaptation to specific host environments. Further genomic analysis is necessary to elucidate the precise mechanisms underlying this divergence and its implications for adenovirus epidemiology and pathogenesis.

The table presents the nucleotide sequence and amino acids identity of the RGD region in nine clinical adenovirus samples compared to various reference adenovirus types. The highest similarity scores for each sample are highlighted, as shown in Tables 2 and 3.

The nucleotide and amino acid alignment of the RGD region of the penton gene revealed a high degree of sequence conservation among HAdV-D23, HAdV-D63, and two clinical samples. While nucleotide differences were observed, particularly in the hypervariable regions, the amino acid sequence remained largely conserved, suggesting strong functional constraints.

The presence of a few amino acid variations in the RGD loop region of the clinical samples may indicate potential recombination events or unique adaptations. Further analysis is required to elucidate the functional implications of these sequence variations and their impact on viral pathogenesis and host interactions, as illustrated in Figures 4, 5, 6, and 7.

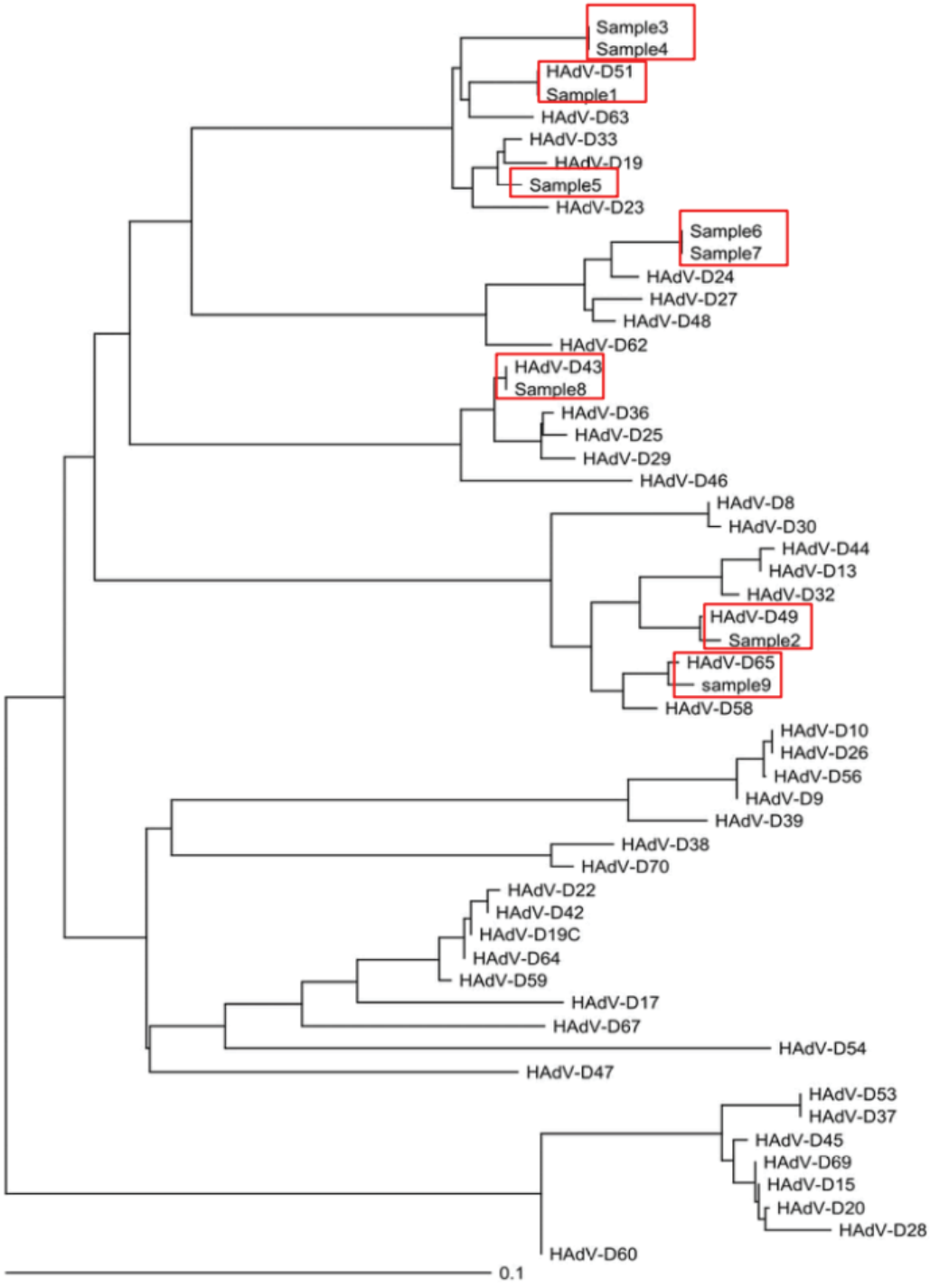


Figure 2. Nucleotide phylogenetic tree of all clinical isolates. The tree was built from the penton RGD region from prototype reference sequences and isolates sequences.

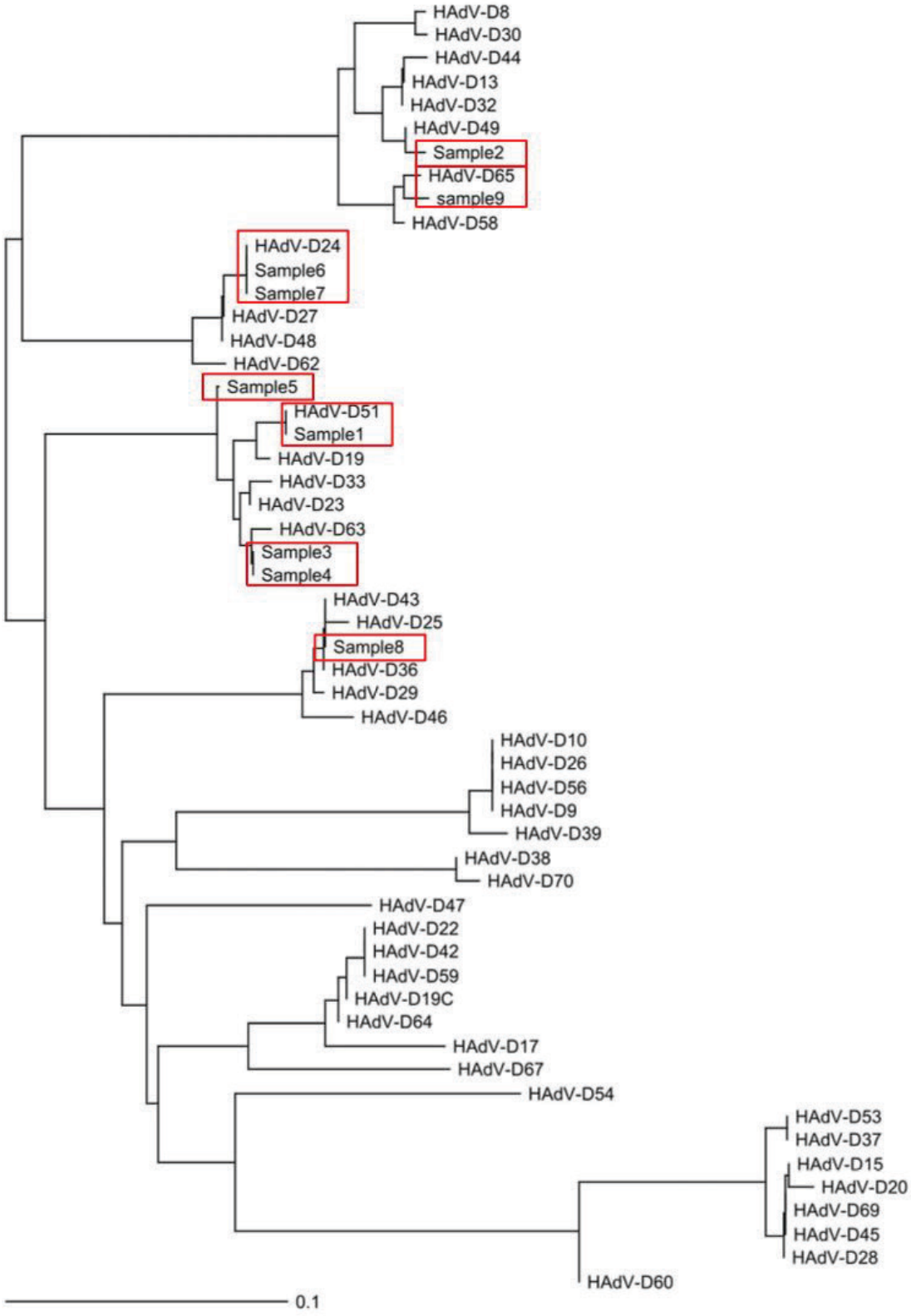


Figure 3. Amino acid phylogenetic tree of all clinical isolates. The tree was built from the penton RGD region from prototype reference sequences and isolates sequences.

Table 2. Nucleotide sequences identity of samples 1-9 RGD region comparing to adenovirus references. Types with highest similarity are highlighted.

Sample8	Sample1	Sample5	Sample4	Sample3	Sample7	Sample6	sample9	Sample2	Seq->
0.805	0.818	0.801	0.813	0.813	0.808	0.808	0.795	0.793	HAdV-D22
0.807	0.82	0.803	0.816	0.816	0.811	0.811	0.797	0.795	HAdV-D42
0.793	0.808	0.791	0.804	0.804	0.794	0.794	0.783	0.781	HAdV-D19C
0.793	0.808	0.791	0.804	0.804	0.794	0.794	0.783	0.781	HAdV-D64
0.814	0.827	0.81	0.818	0.818	0.816	0.816	0.802	0.8	HAdV-D59
0.789	0.806	0.784	0.797	0.797	0.787	0.787	0.781	0.781	HAdV-D17
0.789	0.813	0.791	0.792	0.792	0.801	0.801	0.788	0.783	HAdV-D67
0.779	0.764	0.756	0.766	0.766	0.778	0.778	0.741	0.744	HAdV-D54
0.803	0.778	0.761	0.773	0.773	0.757	0.757	0.755	0.753	HAdV-D10
0.803	0.778	0.761	0.773	0.773	0.757	0.757	0.755	0.753	HAdV-D26
0.803	0.778	0.761	0.773	0.773	0.754	0.754	0.758	0.755	HAdV-D56
0.808	0.783	0.766	0.778	0.778	0.759	0.759	0.762	0.76	HAdV-D9
0.822	0.787	0.766	0.778	0.778	0.766	0.766	0.769	0.765	HAdV-D39
0.803	0.794	0.777	0.785	0.785	0.792	0.792	0.783	0.781	HAdV-D38
0.81	0.799	0.782	0.794	0.794	0.799	0.799	0.79	0.788	HAdV-D70
0.829	0.811	0.803	0.808	0.808	0.778	0.778	0.753	0.76	HAdV-D47
0.802	0.79	0.767	0.781	0.781	0.795	0.795	0.937	0.951	HAdV-D8
0.8	0.79	0.767	0.779	0.779	0.793	0.793	0.934	0.948	HAdV-D30
0.797	0.772	0.755	0.781	0.781	0.783	0.783	0.944	0.958	HAdV-D44
0.8	0.774	0.758	0.783	0.783	0.786	0.786	0.946	0.96	HAdV-D13
0.802	0.781	0.765	0.79	0.79	0.788	0.788	0.948	0.962	HAdV-D32
0.816	0.786	0.769	0.786	0.786	0.788	0.788	0.958	0.995	HAdV-D49
0.811	0.797	0.779	0.786	0.786	0.8	0.8	0.993	0.955	HAdV-D65
0.82	0.795	0.779	0.79	0.79	0.8	0.8	0.979	0.96	HAdV-D58
0.801	0.856	0.842	0.839	0.839	0.98	0.98	0.793	0.786	HAdV-D24
0.801	0.854	0.839	0.846	0.846	0.964	0.964	0.79	0.788	HAdV-D27
0.803	0.858	0.844	0.842	0.842	0.968	0.968	0.8	0.793	HAdV-D48
0.827	0.873	0.846	0.856	0.856	0.952	0.952	0.816	0.804	HAdV-D62
0.848	0.968	0.961	0.954	0.954	0.849	0.849	0.79	0.776	HAdV-D33
0.843	0.971	0.956	0.947	0.947	0.842	0.842	0.79	0.776	HAdV-D19
0.831	0.956	0.947	0.961	0.961	0.844	0.844	0.781	0.772	HAdV-D23
0.843	1	0.937	0.959	0.959	0.844	0.844	0.795	0.781	HAdV-D51
0.845	0.973	0.944	0.959	0.959	0.846	0.846	0.802	0.793	HAdV-D63
1	0.843	0.829	0.831	0.831	0.799	0.799	0.809	0.811	HAdV-D43
0.985	0.831	0.815	0.817	0.817	0.794	0.794	0.802	0.804	HAdV-D36
0.983	0.829	0.813	0.815	0.815	0.792	0.792	0.8	0.802	HAdV-D25
0.981	0.829	0.813	0.815	0.815	0.792	0.792	0.8	0.802	HAdV-D29
0.95	0.808	0.792	0.806	0.806	0.785	0.785	0.781	0.786	HAdV-D46
0.768	0.761	0.747	0.752	0.752	0.721	0.721	0.737	0.737	HAdV-D53
0.768	0.761	0.747	0.752	0.752	0.721	0.721	0.737	0.737	HAdV-D37
0.672	0.691	0.711	0.684	0.684	0.65	0.65	0.648	0.648	HAdV-D60

Table 3. Amino acid sequences identity of samples 1-9 RGD compared to adenovirus reference types. Types with the highest similarity are highlighted.

Sample8	Sample5	Sample1	Sample4	Sample3	Sample7	Sample6	sample9	Sample2	Seq->
0.783	0.807	0.821	0.828	0.828	0.828	0.828	0.77	0.77	HAdV-D22
0.783	0.807	0.821	0.828	0.828	0.828	0.828	0.77	0.77	HAdV-D42
0.783	0.807	0.821	0.828	0.828	0.828	0.828	0.77	0.77	HAdV-D59
0.776	0.8	0.814	0.821	0.821	0.821	0.821	0.763	0.763	HAdV-D64
0.762	0.785	0.8	0.807	0.807	0.807	0.807	0.75	0.756	HAdV-D17
0.755	0.8	0.807	0.807	0.807	0.792	0.792	0.75	0.75	HAdV-D67
0.763	0.756	0.777	0.777	0.777	0.777	0.777	0.93	0.958	HAdV-D8
0.763	0.756	0.777	0.777	0.777	0.777	0.777	0.93	0.958	HAdV-D30
0.777	0.743	0.756	0.763	0.763	0.777	0.777	0.951	0.972	HAdV-D44
0.784	0.75	0.763	0.77	0.77	0.784	0.784	0.958	0.979	HAdV-D13
0.784	0.75	0.763	0.77	0.77	0.784	0.784	0.958	0.979	HAdV-D32
0.784	0.75	0.763	0.77	0.77	0.777	0.777	0.958	0.993	HAdV-D49
0.77	0.756	0.77	0.77	0.77	0.777	0.777	0.986	0.951	HAdV-D65
0.77	0.756	0.77	0.777	0.777	0.784	0.784	0.986	0.958	HAdV-D58
0.776	0.828	0.842	0.85	0.85	1	1	0.777	0.77	HAdV-D24
0.783	0.835	0.85	0.857	0.857	0.992	0.992	0.784	0.777	HAdV-D27
0.783	0.835	0.85	0.857	0.857	0.992	0.992	0.784	0.777	HAdV-D48
0.783	0.828	0.842	0.85	0.85	0.971	0.971	0.791	0.784	HAdV-D62
0.832	0.95	0.971	0.992	0.992	0.842	0.842	0.77	0.763	HAdV-D63
0.825	0.957	0.964	0.985	0.985	0.842	0.842	0.756	0.75	HAdV-D33
0.832	0.964	0.971	0.992	0.992	0.85	0.85	0.763	0.756	HAdV-D23
0.825	0.942	1	0.978	0.978	0.842	0.842	0.763	0.756	HAdV-D51
0.832	0.957	0.985	0.978	0.978	0.835	0.835	0.756	0.75	HAdV-D19
1	0.825	0.825	0.825	0.825	0.776	0.776	0.763	0.777	HAdV-D43
0.993	0.818	0.818	0.818	0.818	0.769	0.769	0.756	0.77	HAdV-D25
1	0.825	0.825	0.825	0.825	0.776	0.776	0.763	0.777	HAdV-D36
0.993	0.825	0.825	0.825	0.825	0.776	0.776	0.763	0.777	HAdV-D29
0.979	0.811	0.811	0.811	0.811	0.762	0.762	0.756	0.77	HAdV-D46
0.804	0.767	0.762	0.769	0.769	0.748	0.748	0.756	0.756	HAdV-D10
0.804	0.767	0.762	0.769	0.769	0.748	0.748	0.756	0.756	HAdV-D26
0.804	0.767	0.762	0.769	0.769	0.748	0.748	0.756	0.756	HAdV-D56
0.804	0.767	0.762	0.769	0.769	0.748	0.748	0.756	0.756	HAdV-D9
0.804	0.76	0.755	0.762	0.762	0.741	0.741	0.75	0.75	HAdV-D39
0.79	0.785	0.774	0.774	0.774	0.788	0.788	0.756	0.77	HAdV-D38
0.783	0.778	0.767	0.767	0.767	0.781	0.781	0.75	0.763	HAdV-D70
0.839	0.821	0.814	0.835	0.835	0.785	0.785	0.722	0.729	HAdV-D47
0.79	0.771	0.778	0.778	0.778	0.778	0.778	0.701	0.708	HAdV-D54
0.734	0.704	0.69	0.697	0.697	0.69	0.69	0.694	0.701	HAdV-D69
0.734	0.704	0.69	0.697	0.697	0.69	0.69	0.694	0.701	HAdV-D45
0.657	0.705	0.671	0.671	0.671	0.657	0.657	0.659	0.666	HAdV-D60

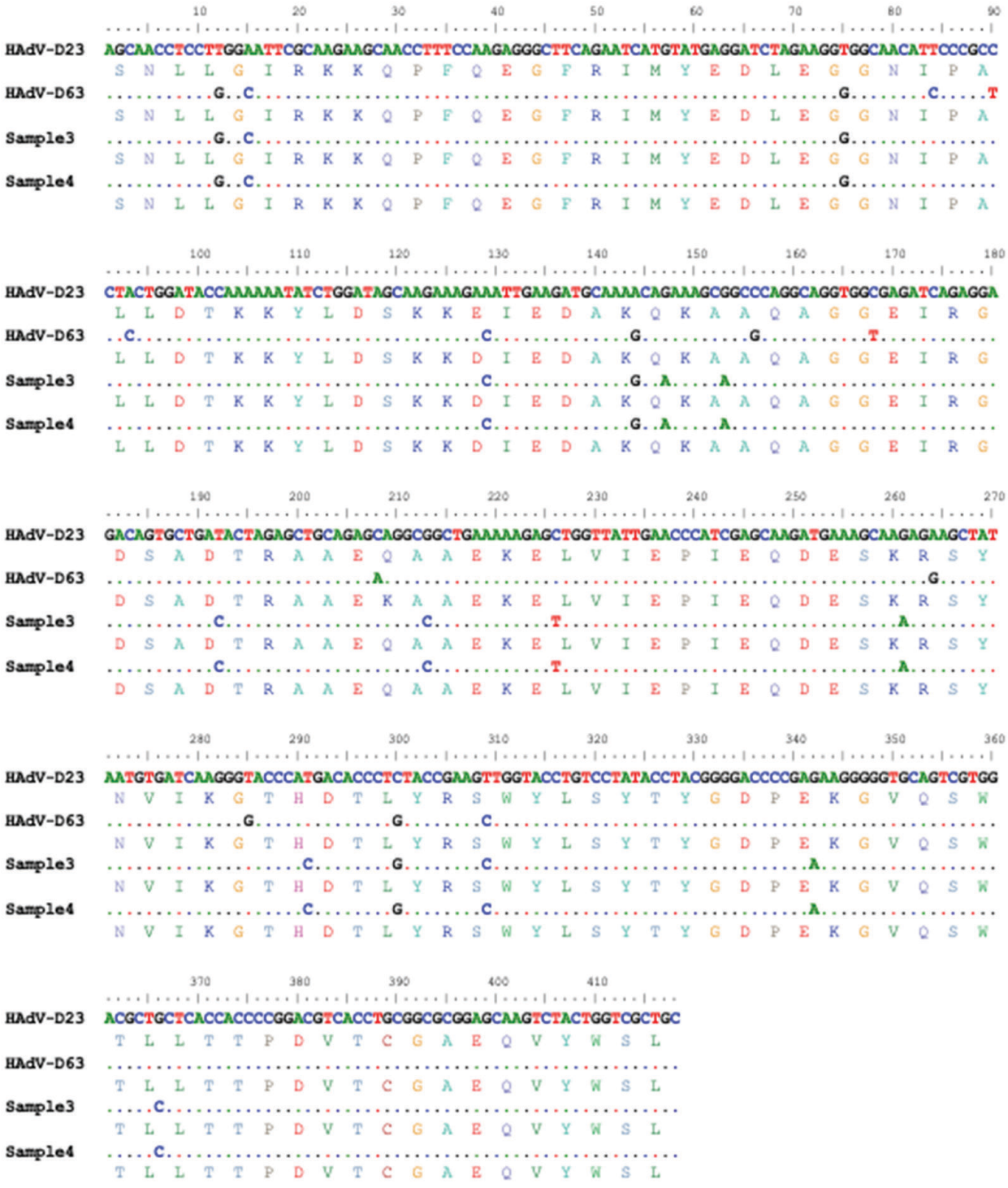


Figure 4. **Nucleotide and amino acid alignment of the RGD region of the penton of HAdV-D 19, 48, and 49 with samples 3 and 4.**

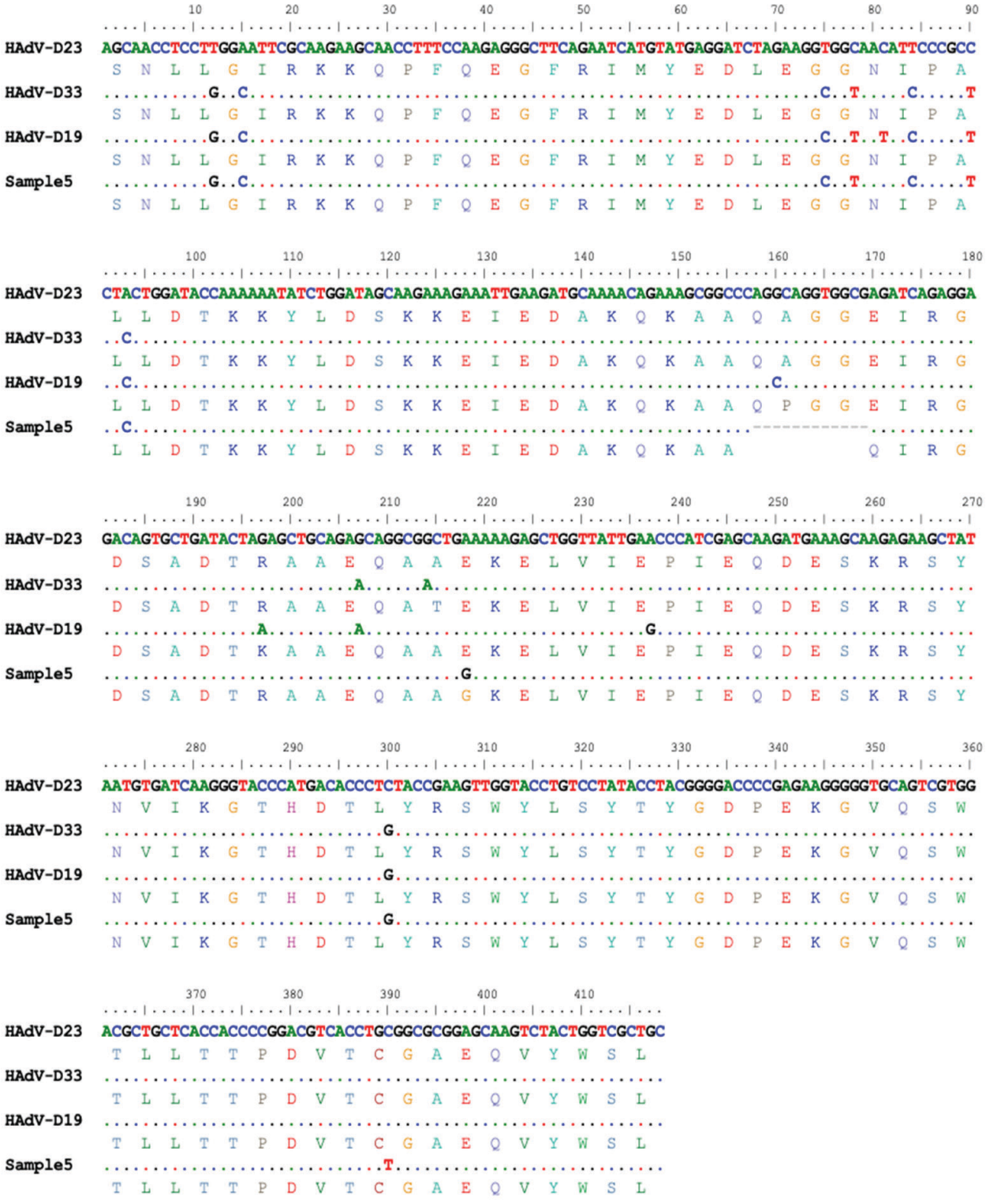


Figure 5. **Nucleotide and amino acid alignment of the RGD region of the penton of HAdV-D 13, 32, 44, 51, and 59 with samples 5.**

Figure 6. Nucleotide and amino acid alignment of the RGD region of the penton of HAdV-D 24 with sample 7.

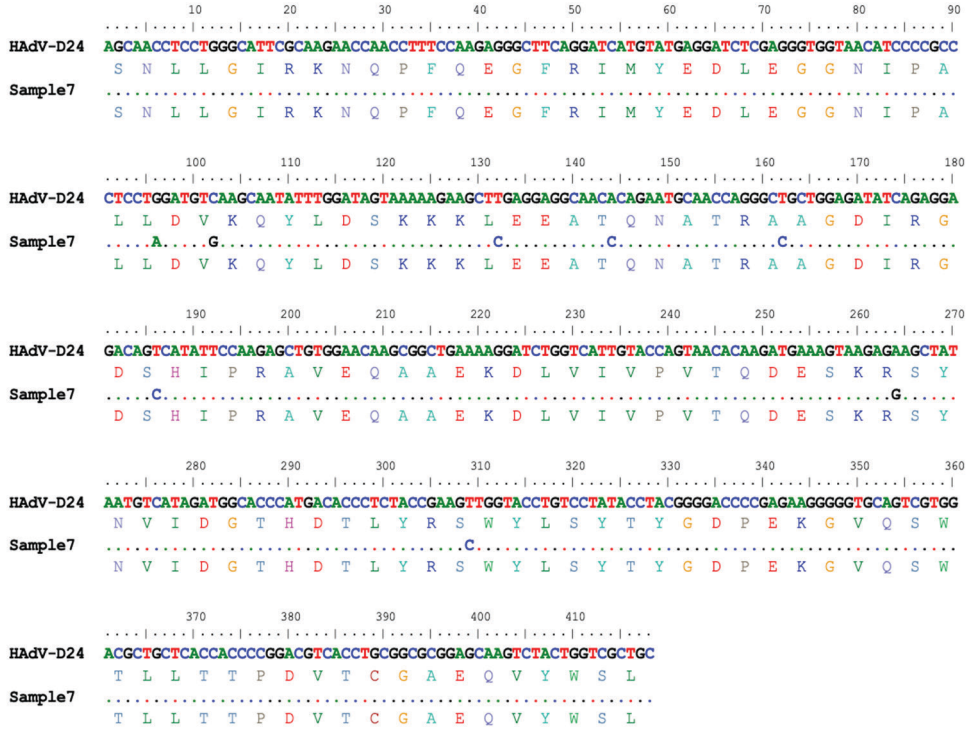


Figure 7. Nucleotide and amino acid alignment of the RGD region of the penton of HAdV-D 9, 10, 47, and 56 with samples 9.

DISCUSSION

The novelty of this study lies in its comprehensive analysis of the penton gene across multiple adenovirus isolates, revealing new insights into adenovirus genetic diversity, especially within species HAdV-D, in immunocompromised patients. Unlike traditional typing that focuses only on the hexon and fiber genes, this study includes an investigation into the penton gene's genetic diversity, identifying recombination events and distinct clades. Such findings suggest that limited-region typing approaches may overlook critical genomic diversity and misclassify adenovirus strains. This study not only detects isolates that do not align with known HAdV types, suggesting novel or recombinant strains, but also underscores the divergence within the penton gene's RGD loop, a functionally significant region that interacts with host cells. The discovery of conserved and variable regions within the RGD loop highlights both evolutionary pressures and possible host-specific adaptations among clinical isolates, insights particularly relevant in immunocompromised individuals. Furthermore, the alignment of nucleotide and amino acid sequences identifies potential new adaptations and recombination events in clinical samples, underscoring the study's importance for understanding adenovirus evolution, pathogenicity, and epidemiology. These findings collectively emphasize the need for whole-genome sequencing and advanced molecular techniques to better characterize adenovirus diversity and evolution, contributing valuable knowledge to the field of adenovirus research and its application in developing adenovirus-based vectors. This study's approach and novel insights represent a meaningful advancement in comprehending adenovirus pathogenesis, especially in vulnerable populations.

The antigenic properties of human adenoviruses are primarily determined by the hexon, fiber, and penton proteins. These capsid proteins are hypervariable regions, which are prone to amino acid substitutions, driving antigenic diversity[18]. However, species D adenoviruses exhibit a unique evolutionary dynamic, characterized by a high rate of homologous recombination[19]. This genetic exchange can lead to the emergence of novel adenovirus types with altered biological properties, including virulence, tropism, and transmissibility. For example, HAdV-D53 and HAdV-D70 are notable examples of recombinant viruses that have arisen through complex recombination events involving multiple parental strains, some of which were previously unknown[20, 21]. These findings highlight the significant impact of recombination on the evolution and diversity of human adenoviruses.

In this study, we conducted a comprehensive analysis of the penton gene in nine species of D adenovirus isolates obtained from individuals with AIDS. These isolates had previously been characterized based on their hexon and fiber gene sequences, displaying identical typing results. To delve deeper into the potential for recombination events, we sequenced and analyzed the penton gene. By comparing these sequences to a reference dataset of 70 human adenovirus types obtained from GenBank, we constructed phylogenetic trees to elucidate evolutionary relationships.

Our analysis of the partial penton region, which encompasses the critical RGD loop, revealed a striking pattern of genetic diversity. Only three of the nine isolates (22, 23, and 24 exhibited consistent typing results across all three structural genes: hexon, fiber, and penton. The remaining six isolates displayed discordant penton types, suggesting the occurrence of recombination events involving different parental strains. Furthermore, we observed identical sequences within the analyzed region for samples 3 and 4, as well as samples 6 and 7, indicating potential clonal relationships or recent transmission events.

These findings highlight the complex evolutionary dynamics of species D adenoviruses and emphasize the importance of considering multiple genetic markers for accurate typing and characterization. Further investigation is warranted to elucidate the mechanisms underlying the observed genetic diversity and its potential impact on viral pathogenesis and disease severity.

The study revealed frequent recombination events within species D adenoviruses, including exchanges between penton and hexon/fiber genes. This genomic instability, particularly in immunocompromised individuals, likely arises from prolonged viral shedding in the gastrointestinal tract[25]. As species D adenoviruses are considered for gene therapy[26], understanding their evolutionary dynamics is crucial to prevent unintended consequences from recombination with viral vectors.

Our findings underscore the significant limitations inherent in traditional adenovirus typing methods, which often rely solely on analyzing specific regions

like the hexon and fiber genes. These conventional techniques, while useful to a degree, fail to capture the full genomic complexity and potential for recombination within the adenovirus genome. As our study has shown, adenoviruses can undergo recombination events not only within hexon and fiber genes but also involving other critical genes like the penton. These recombinations can lead to substantial genetic variability, making traditional, limited-region typing methods unreliable for comprehensive adenovirus classification. The insights from this research highlight the importance of adopting full genome sequencing in adenovirus studies. By sequencing the entire genome, researchers can gain a more complete understanding of the viral genetic structure, identify novel recombinant strains, and reveal hidden diversity that would otherwise be overlooked. This approach is particularly essential for accurately characterizing and monitoring adenoviruses, especially in immunocompromised populations where diverse adenovirus strains can lead to serious health outcomes. In addition, full genome sequencing enables a deeper understanding of the virus's evolutionary patterns, pathogenic potential, and ability to adapt to different host environments. Our findings advocate for a shift toward full genome sequencing as a standard practice in adenovirus research, emphasizing that a broader, more detailed genomic perspective is necessary for accurate characterization, epidemiological tracking, and the development of targeted therapies or vaccines.

Based on the findings of this study, future research could further enhance our understanding of adenovirus genetic diversity and recombination patterns by expanding sequencing efforts beyond specific regions and focusing on full-genome sequencing across a larger sample of adenovirus strains. This would allow for a more comprehensive analysis of recombination hotspots across the genome and may reveal novel regions involved in recombination events.

Additionally, exploring the functional implications of genetic variability, especially within the hypervariable regions and RGD loop of the penton gene, could offer insights into adenovirus-host interactions and pathogenesis. In-depth studies investigating how these genetic changes affect virus fitness, infectivity, and immune evasion could provide critical data for the design of more effective adenovirus-based vectors.

Another promising area for future work would be a comparative analysis of adenovirus recombination patterns across different species, including non-human adenoviruses, to understand how host-specific adaptations influence viral evolution. Finally, research on developing more sophisticated bioinformatic tools to predict recombination events and genetic stability within adenoviruses would be invaluable. These advancements in both methodology and analytical tools would contribute to more accurate adenovirus classification, ultimately informing the development of safer, more effective adenovirus-based therapeutics and vaccines.

In conclusion, this study highlights the limitations of traditional adenovirus typing methods, which focus on partial sequencing of hexon and fiber genes, and underscores the prevalence of recombination events throughout the adenovirus genome. Our findings demonstrate that recombination within the penton gene, particularly in the hypervariable and RGD loop regions, contributes to significant genetic diversity among species D adenoviruses. These insights emphasize the importance of full-genome sequencing for precise adenovirus classification and understanding evolutionary dynamics. This comprehensive approach is essential for advancing adenovirus-based therapies and ensuring the stability and efficacy of adenovirus vectors in clinical applications.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare. All co-authors have seen and agreed with the manuscript's contents, and there is no financial interest to report. We certify that the submission is an original work and is not under review at any other publication.

DISCLOSURE

The authors did not receive any form of commercial support, either in the form of compensation or financial assistance, for this case report. The authors have no financial interest in any of the products, devices, or drugs mentioned in this article.

REFERENCES CITED

[1] Kovács E. 2011. Siadenoviruses: a comparative olecular and phylogenetic study. Budapest: Eotvos Lorand University.

[2] Syyam A, Nawaz A, Ijaz A, Sajjad U, Fazil A, Irfan S, Muzaffar A, Shahid M, Idrees M, and Malik K. (2022). Adenovirus vector system: construction, history and therapeutic applications. Biotechniques, 73(6), 297-305.

[3] Lynch III JP, and Kajon AE. (2021, December). Adenovirus: epidemiology, global spread of novel types, and approach to treatment. In Seminars in respiratory and critical care medicine (Vol. 42, No. 06, pp. 800-821). Thieme Medical Publishers, Inc..

[4] Takuissu G, Kenmoe S, Ebogo-Belobo J, Kengne-Ndé C, Mbaga D, Bowo-Ngandji A, Ondigui Ndzie J, Kenfack-Momo R, Tchatchouang S, and Kenfack-Zanguim J. (2024). Exploring adenovirus in water environments: A systematic review and meta-analysis. International Journal of Environmental Health Research, 34(6), 2504-2516.

[5] Kulanayake S, and Tikoo SK. (2021). Adenovirus core proteins: structure and function. Viruses, 13(3), 388.

[6] Al-Heeti OM, Cathro HP, and Ison MG. (2022). Adenovirus infection and transplantation. Transplantation, 106(5), 920-927.

[7] Gu J, Su Q-q, Zuo T-t, and Chen Y-b. (2021). Adenovirus diseases: a systematic review and meta-analysis of 228 case reports. Infection, 49, 1-13.

[8] Grebe, UF, and Suomalainen M. (2022). Adenovirus entry: Stability, uncoating, and nuclear import. Molecular Microbiology, 118(4), 309-320.

[9] Karabulut OC, Karpuzcu BA, Türk E, Ibrahim AH, and Süzek BE. (2021). ML-AdVInfect: a machine-learning based adenoviral infection predictor. Frontiers in Molecular Biosciences, 8, 647424.

[10] Ricobaraza A, Gonzalez-Aparicio M, Mora-Jimenez L, Lumbreras S, and Hernandez-Alcoceba R. (2020). High-capacity adenoviral vectors: expanding the scope of gene therapy. International Journal of Molecular Sciences, 21(10), 3643.

[11] Nestić D, Božinović K, Pehar I, Wallace R, Parker AL, and Majhen D. (2021). The revolving door of adenovirus cell entry: not all pathways are equal. Pharmaceutics, 13(10), 1585.

[12] Zubieta C, Blanchoin L, and Cusack S. (2006). Structural and biochemical characterization of a human adenovirus 2/12 penton base chimera. The FEBS journal, 273(18), 4336-4345.

[13] Liu L, Qian Y, Jia L, Dong H, Deng L, Huang H, Zhao L, and Zhu R. (2021). Genetic diversity and molecular evolution of human adenovirus serotype 41 strains circulating in Beijing, China, during 2010–2019. Infection, Genetics, and Evolution, 95, 105056.

[14] Cai R, Mao N, Dai J, Xiang X, Xu J, Ma Y, Li Z, Han G, Yu D, and Yin J. (2020). Genetic variability of human adenovirus type 7 circulating in mainland China. PLoS One, 15(4), e0232092.

[15] Hang J, Kajon AE, Graf PC, Berry IM., Yang Y, Sanborn MA, Fung CK, Adhikari A, Balansay-Ames MS., and Myers CA. (2020). Human adenovirus type 55 distribution, regional persistence, and genetic variability. Emerging Infectious Diseases, 26(7), 1497.

[16] Robinson CM., Seto D, Jones MS, Dyer DW, and Chodosh J. (2011). Molecular evolution of human species D adenoviruses. Infect Genet Evol, 11(6), 1208-1217. https://doi.org/10.1016/j.meegid.2011.04.031

[17] Wu X, Zhang J, Lan W, Quan L, Ou J, Zhao W, Wu J, Woo PC, Seto D, and Zhang Q. (2022). Molecular typing and rapid identification of human adenoviruses associated with respiratory diseases using universal PCR and sequencing primers for the three major capsid genes: penton base, hexon, and fiber. Frontiers in Microbiology, 13, 911694.

[18] Madisch I, Hofmayer S, Moritz C, Grintzalis A, Hainmueller J, Pring-Akerblom P, and Heim A. (2007) Phylogenetic analysis and structural predictions of human adenovirus penton proteins as a basis for tissue-specific adenovirus vector design. Journal of Virology 81, 8270-8281.

[19] Robinson CM., Singh G, Lee JY, Dehghan S, Rajaiya J, Liu EB, Yousuf MA, Betensky RA, Jones MS, Dyer DW, and Seto D. (2013). Molecular evolution of human adenoviruses. Scientific reports, 3.

[20] Walsh MP, Chintakuntlawar A, Robinson CM, Madisch I, Harrach B, Hudson NR, Schnurr D, Heim A, Chodosh J, Seto D, and Jones MS. (2009). Evidence of molecular evolution driven by recombination events influencing tropism in a novel human adenovirus that causes epidemic keratoconjunctivitis. PloS one, 4(6), p.e5635.

[21] Hage E, Liebert UG, Bergs S, Ganzenmueller T, and Heim A. (2015). Human mastadenovirus type 70: a novel, multiple recombinant species D mastadenovirus isolated from diarrhoeal faeces of a haematopoietic stem cell transplantation recipient. Journal of General Virology, 96(9), pp.2734-2742.

[22] Rowe WP, Huebner RJ, Hartley JW, Ward TG, and Parrott RH. (1955). Studies of the Adenoidal-Pharyngeal-Conjunctival (APC) Group of Viruses. American Journal of Hygiene, 61(2), pp.197-218.

[23] Davison AJ, Benkő M, and Harrach B. (2003). Genetic content and evolution of adenoviruses. Journal of General Virology, 84(11), 2895-2908.

[24] Davison AJ, Benkő M, and Harrach B. (2003). Genetic content and evolution of adenoviruses. Journal of General Virology, 84(11), 2895-2908.

[25] Curlin ME, Huang ML, Lu X, Celum CL, Sanchez J, Selke S, Baeten JM, Zuckerman RA, Erdman DD, and Corey L. (2010). Frequent detection of human adenovirus from the lower gastrointestinal tract in men who have sex with men. PloS one, 5(6), p.e11321.

[26] Robinson CM, Seto D, Jones MS, Dyer DW, and Chodosh J. (2011). Molecular evolution of human species D adenoviruses. Infection, Genetics and Evolution, 11(6), pp.1208-1217.

ORIGINAL ARTICLE

Modeling Discrete-Event Simulations Using Natural Language Processing: A Healthcare Application

Adnan Miski[1], Asim T. Sharif[2], Ahmed G. Bukhari[3], and Mazen Ismail[2]

[1]*Department of Industrial Engineering, Faculty of Engineering, Rabigh, King Abdulaziz University, Jeddah, Saudi Arabia*
[2]*Department of Medical Education, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia*
[3]*Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia*

Correspondence
Adnan Miski
Department of Industrial Engineering, Faculty of Engineering—Rabigh, King Abdulaziz University, P.O. Box 80205, Jeddah 21589
Kingdom of Saudi Arabia
e-M: agmiski@kau.edu.sa

Submission: 01 Nov. 2024
Accepted: 06 Dec. 2024

Citation
Miski A, Sharif AT, Bukhari AG, and Ismail M. Modeling discrete-event simulations using natural language processing: A healthcare application. JKAU Med Sci 2024; 31(2): 35-47. DOI: 10.4197/Med.31-2.4.

Abstract

Discrete-event simulation (DES) is widely used to model complex healthcare systems; however, manually developing these simulation models often requires extensive effort and specialized expertise. This study explored how natural language processing (NLP) techniques can automate DES model generation from text descriptions and optimize resource allocation in the healthcare domain. We used the GPT-4o large language model to demonstrate that DES models can be automatically generated from natural language prompts with accuracy comparable to traditional simulation software. The GPT-4o model successfully simulated a skin care clinic and a complex medical care facility, producing results aligned with Arena software for metrics such as average queue times and patient throughput. Additionally, GPT-4o determined the optimal resource allocation to minimize costs while satisfying the patient waiting time constraints. The automated generation of simulations shows the potential to combine NLP with DES to accelerate healthcare system modeling and optimization.

Keywords

Discrete-event simulation, Natural language processing, Healthcare simulation automation, Resource allocation optimization, Decision support systems

INTRODUCTION

Healthcare systems are complex and dynamic, requiring sophisticated modeling approaches to analyze and optimize their performance. Discrete-event simulation (DES) has emerged as a powerful tool for simulating healthcare processes, allowing researchers and practitioners to evaluate various scenarios and resource allocation strategies without disrupting real-world operations. However, the development of DES models traditionally requires specialized knowledge and can be time-consuming, limiting accessibility and widespread adoption in healthcare decision-making[1]. Recent advances in artificial intelligence (AI) and natural language processing (NLP) offer promising opportunities for streamlining and automating the DES model generation process. By leveraging language models trained on large volumes of data, there is the potential to create validated simulation models directly from the natural language descriptions of healthcare systems. This approach could significantly reduce the technical barriers to entry into DES modeling and enable the rapid exploration of multiple scenarios.

This study investigates the potential of using NLP to automate the generation of DES models for healthcare applications. We explore two research questions: (i) How can simulation engineers automatically generate DES models for healthcare systems from natural language descriptions? (ii) How can NLP techniques be used to interpret the results of these simulations to optimize resource allocation in complex healthcare systems? This study aims to streamline the development of simulation models and enhance decision-making capabilities through the automated analysis of simulation outputs. By exploring these questions, this study contributes to the growing field of AI applications in healthcare simulations. The findings may have significant implications for improving the accessibility and efficiency of healthcare system modeling, which could lead to better-informed decisions and improved system outcomes.

The article is structured as follows: Section 2 reviews related work and examines previous applications of DES and NLP in healthcare. Section 3 describes the background and methodology, detailing the language models and frameworks used to generate and evaluate simulation models. Section 4 presents the experimental setup and results and compares the performance of GPT-4o with traditional simulation software. Section 5 discusses the findings, focusing on the potential of large language models to automate simulation development and resource allocation. Finally, Section 6 concludes the study by summarizing the key findings and outlining future research directions.

RELATED WORK

DISCRETE-EVENT SIMULATION IN HEALTHCARE

Discrete-event simulation, also known as event-driven or time-to-event simulation, is a powerful technique used to evaluate complex systems and inform decision-making processes. DES is used in several industries, such as manufacturing, logistics, aviation, and healthcare. In healthcare organizations, medical facilities have extensively used DES to enhance operational efficiency, achieve cost savings, and optimize resource allocation[2]. For example, DES has been employed to evaluate the impact of operational policies, such as wait time thresholds, discharge windows, and patient mix combinations, on the behavior and performance of intensive care units (ICUs)[3]. Pradelli et al.[4] used DES to compare the efficacy of parenteral nutrition regimens with and without omega-3 fatty acids, showing that omega-3 supplementation led to cost savings in ICU and non-ICU patients across four countries. Lenin et al.[5] focused on optimizing clinic appointments and staff numbers to reduce patient waiting times using DES. Moreover, Sala et al.[6] utilized DES to analyze the impact of COVID-19 on outpatient healthcare facilities, finding that pandemic-related policies resulted in a significant reduction in the utilization of MRI equipment. Forbus et al.[7] developed a DES model to optimize physician resource allocation in pediatric hospitals, leading to improved operational efficiency and patient care. The simulation effectively modeled patient journeys, enabling the identification of bottlenecks and inefficiencies. The body of research on DES has been expanding due to the ability of this modeling approach to represent complex systems using detailed stochastic factors, which allow for the simulation of variability and uncertainty inherent in real-world processes[2].

NATURAL LANGUAGE PROCESSING-BASED SYSTEMS

NLP involves enabling machines to read, comprehend, and extract meaning from human language. It can be categorized into natural language understanding (NLU), which focuses on comprehending and extracting

meaning from text, and natural language generation (NLG), which focuses on text production[8]. Recent advancements in digitalization and the abundance of textual data have enabled significant breakthroughs in NLP, such as summarization, information extraction, question answering, text generation, and sentiment analysis. Furthermore, specialized tasks such as optimizing optical character recognition (OCR) performance for videos have benefited from NLP advancements, leveraging large language models and image super-resolution for code and text extractions[9]. These technological advancements have enhanced people's lives and substantially transformed decision support and expert systems[10].

NLU is used to comprehend meaning that extends beyond the literal interpretation of individual words, encompassing the comprehension of contextual information, sentiment, and communicative intent[11, 12]. Significant results have been obtained using NLU. For instance, Brito et al.[13] leveraged NLU techniques to forecast election results based on social media data, whereas Kastrati et al.[14] employed NLU to analyze student responses and offer tailored feedback. Lin et al.[15] developed a chatbot using natural language understanding to assist construction site managers with efficient information retrieval by accurately predicting the intents and entities from their inquiries. By analyzing the semantic and pragmatic dimensions of language, NLU can enable machines to engage in more naturalistic and meaningful interactions with humans.

In contrast, NLG develops models that produce coherent, readable, and contextually relevant texts. The objective is to enable machines to automatically generate human-like languages based on data or inputs. Extensive scholarly work has been devoted to exploring NLG applications. For example, García-Méndez et al.[16] developed NLG systems capable of generating human-like text with completeness, grammatical correctness, and semantic coherence. Mulla et al.[17] used NLG to automatically generate factual, multiple-sentence, and yes/no-type questions. Lin et al.[18] applied NLG to generate natural language descriptions from structured data sources such as tables and graphs.

Engaging in both NLU and NLG is a fundamental capability of advanced large language models (LLMs). These models are highly adept at comprehending complex language inputs and generating relevant, coherent textual outputs, making them valuable tools for a wide range of applications.

AI SYSTEMS FOR DISCRETE-EVENT SIMULATION AND RESOURCE ALLOCATION IN HEALTHCARE

Developing a DES model can be time-consuming, relying heavily on high-quality data to accurately represent a system's behavior[2]. The healthcare field has recently witnessed a growing trend of incorporating AI techniques into DES models to accelerate the modeling process and mitigate associated expenses. For instance, Gartner et al.[19] linked machine learning results with a DES model to demonstrate that changing staffing patterns can reduce the overestimation of waiting times, potentially enhancing patient satisfaction. Hosseini-Shokouh et al.[20] focused on optimizing service processes in emergency departments using a combination of DES, artificial neural network algorithms, and genetic algorithms to minimize patient waiting times and improve efficiency. Olave-Rojas et al.[21] presented a hybrid emergency medical services simulation model with a machine learning approach for travel speed forecasting. In a recent study, Atalan et al.[22] integrated DES models with machine learning algorithms to estimate the number of patients treated and predict patient waiting times in healthcare institutions based on healthcare resource costs. In another recent study, Ortiz-Barrios et al.[3] integrated AI and DES to support decision-making for ICU capacity management, offering insights for timely interventions and reducing bed waiting times. These studies demonstrate the potential of leveraging AI techniques to enhance the development and accuracy of discrete-event simulation models, particularly in complex and data-intensive domains such as healthcare.

BACKGROUND AND METHODOLOGY

LANGUAGE MODELS

Language models serve as fundamental building blocks in modern NLP systems. The primary function of these models is to predict the sequence of words or codes in relation to the prior context[11]. For a set of training examples (s_1, s_2, ..., s_n), where each example expresses a sequence of words with varying lengths (w_1, w_2, ..., w_n), the predictive output of the language models can be represented as the product of the conditional probabilities, as shown in Equation (1)[23]:

$$p(s) = \prod_{i=1}^{n} p(w_n | w_1, w_2, \ldots, w_{n-1}) \quad (1)$$

LLMs are trained on large datasets to capture intricate relations between words. State-of-the-art LLMs, including generative pre-trained transformer (GPT), bidirectional encoder representations from transformers (BERT), and Claude, have significantly improved the statistical model in Equation (1) and advanced the field of NLP by demonstrating exceptional performance across a range of diverse applications. Researchers have used these LLMs by prompting models to perform tasks, fine-tuning the models according to interest, or using the models to generate code[11]. This study utilizes a transformer-based language model, specifically the GPT architecture, which demonstrates remarkable efficacy in text, code generation, and understanding.

TRANSFORMER

The transformer architecture has emerged as the foundational backbone for training state-of-the-art LLMs, surpassing the capabilities of recurrent and convolutional neural network architectures. Transformer architectures leverage an attention mechanism (Equation 2), comprising self-attention and feed-forward networks, enabling LLMs to predict parallel sequences that handle long sequences with exceptional efficiency[24].

$$\text{Attention}(Q,K,V) = \text{softmax}\left(\frac{QK^T}{\sqrt{d_k}}\right) \qquad 2$$

where the query (Q), key (K), and value (V) matrices are derived from the input sequence, and the dimensionality of the key vectors is represented by d_k. The softmax function is used to normalize the dot products, which are then used to calculate the model's output[24].

GENERATIVE PRE-TRAINED TRANSFORMER (GPT)

Generative pre-trained transformer models, developed by OpenAI in San Francisco California, employ deep learning to understand and generate natural language. The progression of the GPT family of models, from GPT-1 to GPT-4, has demonstrated remarkable advancements in output quality and accuracy[25]. These GPT models have been widely applied in recent academic research to produce a wide range of outputs, such as text, code classification, and images, to improve the efficiency in specialized domains[26,27]. This study utilized the latest iteration of the GPT model, GPT-4o, a multimodal language model that integrates and processes diverse data types and addresses complex, multi-step tasks[25]. The GPT-4o language model was used to perform DES and resource allocation, to inform and optimize healthcare decision-making.

EXPERIMENT

Prompt engineering has emerged as a crucial practice for fully leveraging the capabilities of GPT models. This practice is characterized by an iterative process of refining text input or prompts to elicit optimal responses from the language model[28]. This process can be accelerated substantially by adhering to the fundamental principles of prompt engineering. One such principle entails providing the model with comprehensive and detailed contextual information before task initiation, thereby ensuring the relevance and accuracy of the model's responses. Another principle involves breaking down complex tasks into smaller, more manageable components, which can be addressed sequentially.

A high-level framework was developed to address the research questions and facilitate the semi-automation of experiments (see Figure 1). This framework shows the language model's ability to translate textual problem descriptions into functional Python-based DESs, which are then executed on the Replit development platform.

The proposed framework comprises seven key elements. First, the process begins with a detailed textual description of the healthcare system including a clear context and task specifications prompt. Second, GPT-4o processes the prompt and applies its deep knowledge of healthcare concepts and advanced programming capabilities. Third, these programming capabilities generate a functionally accurate DES in Python code. Fourth, the generated code is executed in the Replit cloud-based integrated development environment. Fifth, the code produces results that reflect the system's performance and provides valuable insights. Sixth, these outputs are evaluated by a domain expert to ensure the validity and integrity of the findings. Finally, a feedback loop is established, enabling the insights gained from the results and expert assessments to inform refinements of the initial prompt.

SIMULATED HEALTHCARE SYSTEMS

This study examined and simulated two healthcare systems with varying levels of complexity. The first

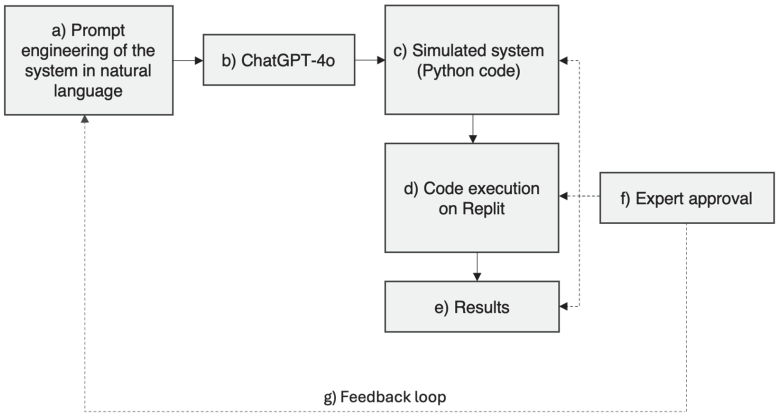


Figure 1. **High-level representation of the proposed framework.**

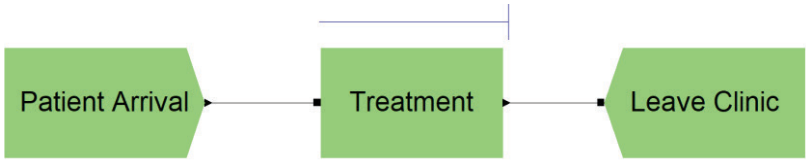


Figure 2. **Skin care clinic model in Arena.**

system was a skin care clinic, and the second was a medical care facility. Each system was evaluated in terms of two problems. The first problem focused on simulating the system operations, and the second problem analyzed the results from the initial simulation to optimize resource allocation. The GPT model was prompted with the first problem, and its results were validated against the traditional simulation software Arena, which is one of the most popular software for complex systems modeling[2]. Subsequently, the GPT model was prompted with the second allocation problem, and its outputs were compared with OptQuest optimization software for validation.

SKIN CARE CLINIC SYSTEM

The skin care clinic model in Arena is shown in Figure 2 and its description is as follows: Patients arrive at a skin care clinic according to an exponential interarrival time distribution with a mean of 16.2 min, with the first arrival at time 0. At the clinic, patients wait in a single first-in first-out line until one of two doctors is available to see them. The treatment process follows a triangular distribution with a minimum of 23, mode of 25, and maximum of 27. A 14-day simulation is run with 100 replications of 14 hours per day, and the number of patients exiting the system and the average waiting time are calculated.

After obtaining the results from the model, it was prompted with the following: What is the optimal number of doctors in the clinic, given that the hourly rate is $30, and the objective is to minimize the total cost while ensuring that the average waiting time for patients does not exceed 5 minutes.

MEDICAL CARE SYSTEM

The medical care facility model in Arena is shown in Figure 3 and its description is as follows: Patients arrive at a medical care facility according to an exponential interarrival time distribution with a mean of 11 min. Upon arrival, a registration receptionist checks in patients, and this process follows a triangular distribution with a minimum of 5 min, a mode of 10 min, and a maximum of 15 min. After registration, patients wait for one of two doctors at the facility. The checkup time follows a triangular distribution with a minimum of 14 min, a mode of 22 min, and a maximum of 39 min. After the checkup, ten percent of the patients may require lab tests before leaving the facility. The lab test is conducted by one nurse, and it follows a triangular distribution with a minimum of 20 min, a mode of 30 min, and a maximum of 40 min. Thirty percent of the patients may require seeing one of two specialists after the examinations. The specialist consultation follows a triangular distribution with a minimum of 25 min, a

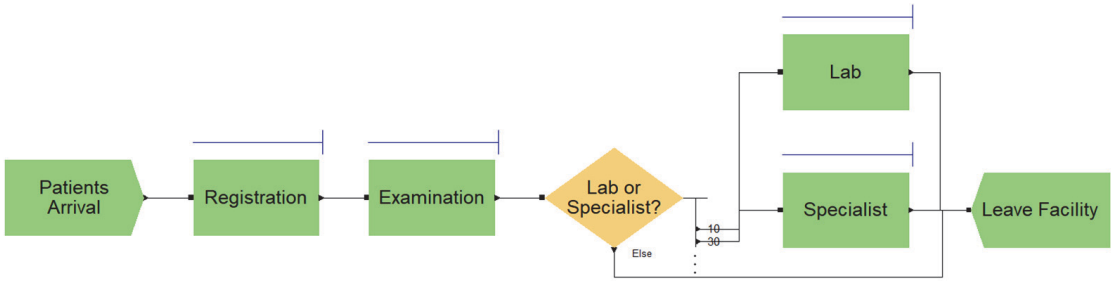


Figure 3. **Medical care facility model in Arena.**

mode of 35 min, and a maximum of 50 min. A discrete-event simulation is run for a duration of 7 days, with 100 replications of 16 hours per day. The number of patients exiting the system and the average time in queue for registration, examination room, lab tests, and consultation are calculated.

After obtaining the results from the model, it was prompted with the following: What is the optimal number of employees in the facility, given that the hourly rate for the receptionist is $15, the nurse $25, the doctor $40, and the specialist $80. The objective is to minimize the total cost while ensuring that the average waiting time for patients does not exceed 10 minutes for each queue.

RESULTS

The experiments are reproducible; the source code files are publicly accessible in the GitHub repository.

SKIN CARE CLINIC SYSTEM RESULTS

The LLM was prompted with a clear English description of the skin care clinic system. It then generated a DES model in Python with a clear explanation of the code and automatically visualized the average waiting time in the queue per replication (see Figure 4). The simulation model initiated the process by importing essential Python libraries required for random number generation and data visualization. The relevant parameters and data structures were then defined. Subsequently, the model correctly implemented the logic underlying DES. Specifically, it initiated patient arrivals at time zero and randomly generated treatment times. Next, the simulation was run with exponential patient arrival times. Finally, the model defined and executed replication steps, appending the average time in queues and the total number of patients exiting the system to a list. Notably, to ensure the validity of the results, the initial prompt should explicitly specify the usage of the SimPy library.

A comparison of the results in Table 1 shows that the simulations generated by the large language model GPT-4o yielded outputs comparable to those produced by the established industrial simulation software Arena. The average time spent in the queue is a crucial metric for assessing the efficiency of simulated healthcare systems. The GPT-4o model reported an average time in queue of 18.31 min, while the Arena software reported 18.06 min. These results suggest that the two models exhibit comparable performance, with Arena demonstrating a marginally lower average time in queue. This minor discrepancy may be attributable to differences in the respective systems' underlying algorithms or optimization techniques. Another key performance indicator is the total number of patients who successfully exited the system, which represents the throughput and capacity of the modeled healthcare system; both GPT-4o and Arena yielded comparable results.

After executing the initial simulation problem prompt and recording the results, the LLM was provided with the resource allocation problem prompt to determine the optimal number of doctors required to reduce patient waiting times and minimize the total operational costs (see Figure 5). Notably, the language model generated visualizations of the average patient waiting time, even though this metric was not specified in the original prompt. These visualizations covered the baseline simulation scenario, as shown in Figure 4, and the optimized levels, as shown in Figure 5. This automated plotting capability may reflect the prevalence of DES samples with associated data visualizations available on platforms such as GitHub[8].

Table 2 presents a comparative performance evaluation of the GPT-4o model and the OptQuest

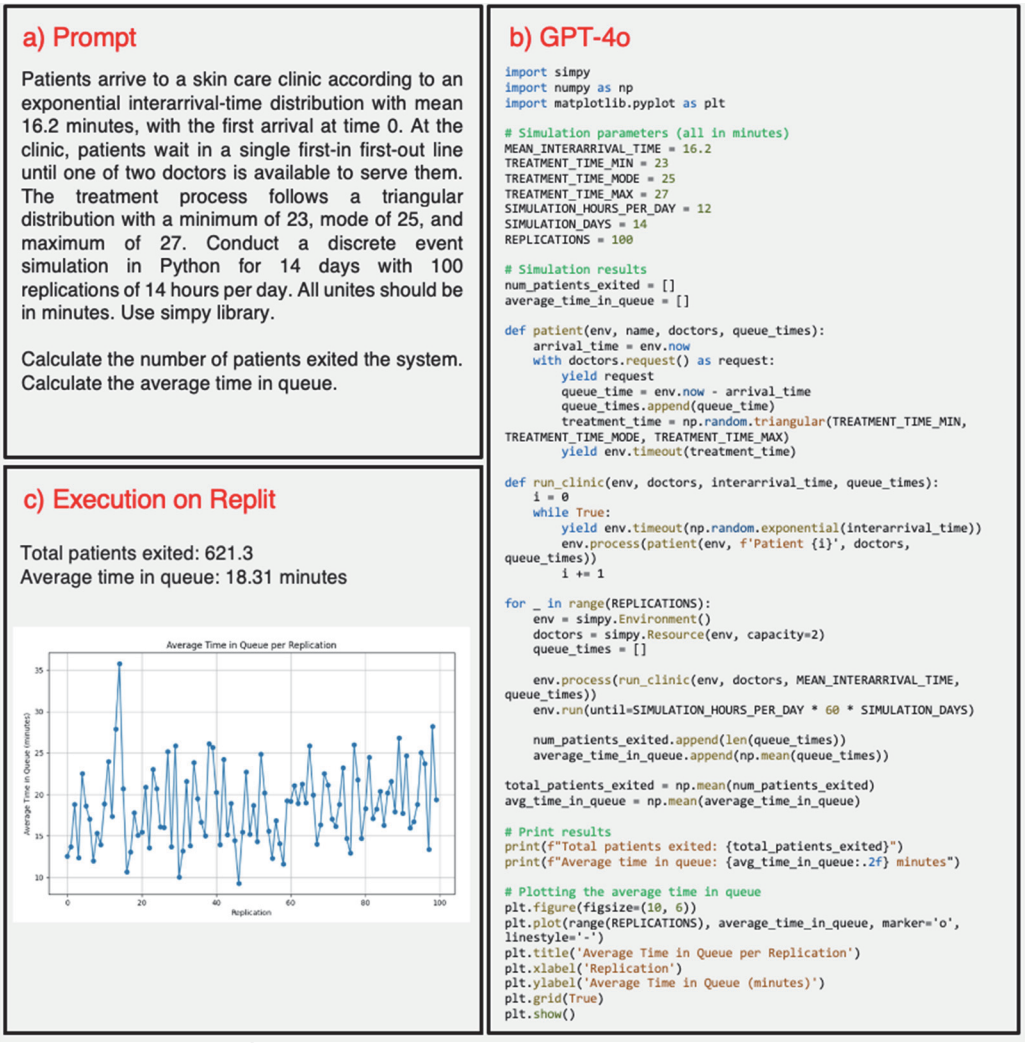


Figure 4. **Skin care system prompt and outputs.**

Table 1. **Comparing GPT-4o and Arena simulation results of the skin care clinic system.**

	GPT-4o	Arena Software
Avg. time in queue (min.)	18.31	18.06
Total patients exited the system	621.3	618

software within Arena, focusing on the average queue time and total cost for the optimal number of doctors. The results show that the average time in queue is comparable between the two approaches, suggesting similar effectiveness in modeling patient flow. While the costs associated with the GPT-4o model were slightly higher than those of Arena, the difference was insignificant, indicating that the GPT-4o model was able to achieve comparable resource optimization. Importantly, both OptQuest and GPT-4o correctly identified three doctors as the optimal staffing level, demonstrating the effectiveness of the automated simulation approach in determining the most efficient resource allocation for the healthcare system.

MEDICAL CARE SYSTEM RESULTS

The LLM generated lengthy Python code in response to the medical care problem prompt, akin to the previous simulation problem. The generated code, which is available in the GitHub repository, includes automated data visualization (see Figure 6). However, the produced code exhibited variable name conflicts. Despite this, the model was prompted again to identify and resolve the naming conflicts.

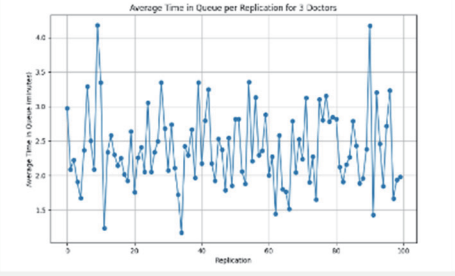
a) Prompt

What is the optimal number of doctors in the clinic, given that the hourly rate is $30, and the objective is to minimize the total cost while ensuring that the average waiting time for patients does not exceed 5 minutes.

c) Execution on Replit

Doctors: 1, Total Cost: $5040.00, Average Queue Time: 1761.01 minutes, Patients Exited: 402.53
Doctors: 2, Total Cost: $10080.00, Average Queue Time: 17.98 minutes, Patients Exited: 615.43
Doctors: 3, Total Cost: $15120.00, Average Queue Time: 2.38 minutes, Patients Exited: 621.40
Doctors: 4, Total Cost: $20160.00, Average Queue Time: 0.49 minutes, Patients Exited: 623.63
Doctors: 5, Total Cost: $25200.00, Average Queue Time: 0.11 minutes, Patients Exited: 618.55

Optimal number of doctors: 3



b) GPT-4o Output

```
DOCTOR_HOURLY_RATE = 30

def calculate_total_cost(num_doctors):
    average_time_in_queue, total_patients_exited,
average_time_in_queue_list = run_simulation(num_doctors)
    cost_of_doctors = num_doctors * DOCTOR_HOURLY_RATE *
SIMULATION_HOURS_PER_DAY * SIMULATION_DAYS
    cost_of_waiting_time = average_time_in_queue *
total_patients_exited * WAITING_TIME_COST_RATE
    total_cost = cost_of_doctors + cost_of_waiting_time
    return total_cost, average_time_in_queue, total_patients_exited,
average_time_in_queue_list

# Find the optimal number of doctors
doctor_range = range(1, 11)  # Testing from 1 to 10 doctors
results = []

for num_doctors in doctor_range:
    total_cost, average_time_in_queue, total_patients_exited,
average_time_in_queue_list = calculate_total_cost(num_doctors)
    results.append((num_doctors, total_cost, average_time_in_queue,
total_patients_exited, average_time_in_queue_list))

# Extracting the optimal number of doctors
optimal_doctors = None
min_cost = float('inf')
for num_doctors, total_cost, average_time_in_queue,
total_patients_exited, average_time_in_queue_list in results:
    if average_time_in_queue <= 5 and total_cost < min_cost:
        min_cost = total_cost
        optimal_doctors = num_doctors

# Print results
for num_doctors, total_cost, average_time_in_queue,
total_patients_exited, average_time_in_queue_list in results:
    print(f"Doctors: {num_doctors}, Total Cost: ${total_cost:.2f},
Average Queue Time: {average_time_in_queue:.2f} minutes, Patients
Exited: {total_patients_exited:.2f}")

print(f"\nOptimal number of doctors: {optimal_doctors}")

# Plotting the average time in queue per replication for the optimal
number of doctors
for num_doctors, total_cost, average_time_in_queue,
total_patients_exited, average_time_in_queue_list in results:
    if num_doctors == optimal_doctors:
        plt.figure(figsize=(10, 6))
        plt.plot(range(REPLICATIONS), average_time_in_queue_list,
marker='o', linestyle='-')
        plt.title(f'Average Time in Queue per Replication for
{num_doctors} Doctors')
        plt.xlabel('Replication')
        plt.ylabel('Average Time in Queue (minutes)')
        plt.grid(True)
        plt.show()
```

Figure 5. **Skin care system resource allocation prompt and outputs.**

Table 2. **Comparison of GPT-4o and OptQuest optimization results of the skin care clinic system.**

	Control (Number of Doctors)	GPT-4o	Arena Software (OptQuest)
Avg. time in queue (min.)	1	1761.01	1794
	2	17.98	18.065
	3	2.38	2.343
Objective function (minimize total cost)	1	$5040.00	$5038.91
	2	$10,080.00	$10,063.58
	3	$15,120.00	$15,104.13

Table 3 shows that while the GPT-4o model generally exhibited shorter average waiting times, GPT-4o and Arena demonstrated comparable performance in terms of overall patient throughput. These findings suggest that the GPT-4o language model is a viable alternative for handling complex simulation problems in healthcare systems. In fact, the GPT-4o model was able to achieve similar levels of patient throughput as the established Arena simulation software, indicating its potential to automate and streamline the simulation development process for healthcare applications.

Following the execution and analysis of the initial simulation scenario, the LLM was presented with a resource allocation prompt. The goal was to leverage the model to determine the optimal staffing

a) Prompt

Patients arrive at a medical care facility according to an exponential interarrival-time distribution with a mean of 11 minutes. Upon arrival, a registration receptionist checks-in patients, and this process follows a triangular distribution with a minimum of 5 minutes, a mode of 10 minutes, and a maximum of 15 minutes. After registration, patients wait for one of two doctors at the facility. The checkup time follows a triangular distribution with a minimum of 14 minutes, a mode of 22 minutes, and a maximum of 39 minutes. After the checkup, ten percent of the patients may require lab tests before leaving the facility. The lab test is done by one nurse, and it follows a triangular distribution with a minimum of 20 minutes, a mode of 30 minutes, and a maximum of 40 minutes. Thirty percent of the patients may require seeing one of two specialists after the examinations. The specialist consultation follows a triangular distribution with a minimum of 25 minutes, a mode of 35 minutes, and a maximum of 50 minutes. Conduct a discrete event simulation in Python for a duration of 7 days, with 100 replications of 16 hours per day. All units should be in minutes. Use simpy library.

Calculate the average time in queue for registration, examination room, lab tests, and consultation.
Calculate the number of patients exited the system.

b) Execution on Replit

Average time in queue for registration:
46.01 minutes
Average time in queue for doctors:
375.10 minutes
Average time in queue for lab tests:
3.06 minutes
Average time in queue for specialists:
2.07 minutes
Average number of patients exited the system:
530.83

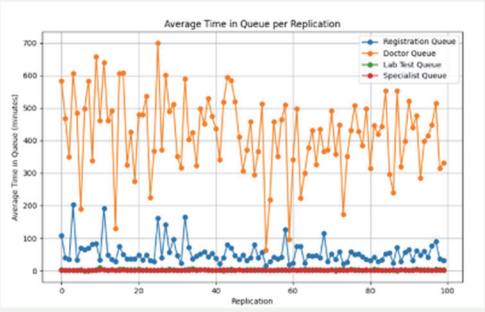


Figure 6. **Medical care system prompt and outputs.**

Table 3. **Comparing GPT-4o and Arena simulation results of the medical care system.**

	GPT-4o	Arena Software
Avg. time in queue for registration (min.)	46.01	46.47
Avg. time in queue for doctors (min.)	375.10	388.02
Avg. time in queue for lab tests (min.)	3.06	2.97
Avg. time in queue for specialists (min.)	2.07	1.755
Total patients exited the system	530.83	531.00

levels of receptionists, doctors, nurses, and specialists required to minimize patient waiting times and overall operational costs (see Figure 7).

The comparative analysis presented in Table 4 shows that the outputs generated by the LLM differed from those generated by OptQuest software. This discrepancy may be attributed to the use of divergent algorithms to calculate the average waiting time in the queue. Nonetheless, the language model successfully determined the optimal allocation of resources, which satisfies the specified objective function and constraints.

In summary, the findings suggest that GPT-4o can execute simulations with varying complexities. The model showed an understanding of the fundamental principles underlying DES, as well as the domain-specific context. Additionally, the LLM determined the optimal allocation of resources and automatically generated appropriate visualizations for the given problems. This outcome indicates that providing the model with only an English-language description of the simulation and resource allocation problem could save simulation engineers significant time and reduce the costs of acquiring traditional high-end simulation software. These results highlight the substantial potential of leveraging LLMs in decision-making processes within the healthcare system and other industries.

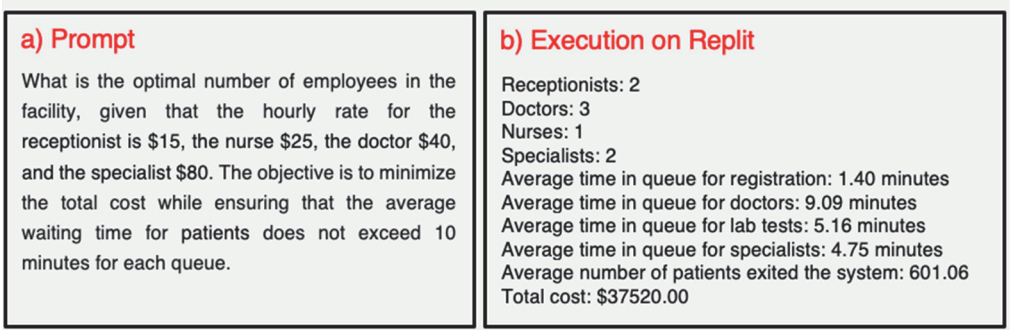


Figure 7. **Medical care system resource allocation prompt and outputs.**

Table 4. **Comparison of GPT-4o and OptQuest optimization results of the medical care system.**

	GPT-4o Optimal Value	Arena Software (OptQuest)
Receptionists	2	2
Doctors	3	3
Nurses	1	2
Specialists	2	2
Total Cost	**$37,520**	**$40,296.64**

DISCUSSION

The results of this study highlight the promising capabilities of GPT-4o in automating and optimizing the generation of DES models for healthcare applications, thereby offering improved efficiency compared to traditional simulation software.

LLMS FOR DISCRETE-EVENT SIMULATION

GPT-4o has demonstrated impressive proficiency in programming with Python, coupled with a comprehensive grasp of simulation and healthcare-specific terminology and concepts. Furthermore, this advanced model exhibits the capacity to generate functionally accurate DESs of healthcare systems based solely on textual descriptions provided in English. Earlier GPT models were limited in handling simple simulation problems due to technical limitations, such as the number of parameters used during training, and the context window size[8,30]. In contrast, GPT-4o can generate simulations for systems with varying degrees of complexity.

Although GPT-4o is a groundbreaking LLM in our study, recent advances have significantly expanded the capabilities of such language models. Meta-AI recently launched Llama, continuing with enhanced efficiency and scalability, designed to meet the demands of complex research applications better[30]. Google introduced the Gemini model, which integrates advanced multimodal capabilities, combining text, images, and other data types, further pushing the boundaries of what language models can achieve[31]. Additionally, Anthropic researchers in San Francisco, California have made considerable progress with Claude, emphasizing improvements in reasoning and language comprehension capabilities, contributing to more reliable and interpretable outputs[30]. These advancements indicate that the magnitude and complexity of simulations generated by the framework proposed in this study could continue to expand, facilitating increasingly sophisticated applications in the future.

Automation of DESs, as highlighted in our research, can reduce simulation time, resources, and costs, and minimize human error during modeling. Conventional simulation software, such as Arena, Simio, or AnyLogic, requires meticulous design, comprehensive calibration, and thorough validation procedures. While these fundamental steps are essential, they can be resource-intensive and prone to human error, particularly when modeling complex systems. In contrast, our approach, which uses the capabilities of the GPT-4o model and the Replit development environment, provides a more efficient and streamlined process that can minimize human error. Furthermore, the adaptability afforded

by leveraging prompt engineering techniques enables expeditious iterations and modifications, thereby transcending the intrinsic rigidity typically associated with conventional simulation software.

Although AI-powered simulation development offers significant advantages, users should recognize that these methods are not intended to replace the expertise of seasoned simulation modelers. As highlighted in this study, minor discrepancies may exist between the outputs of the GPT-4o model and those of established industrial simulation software. In addition, LLMs can generate code with bugs that require an engineer's intervention to rectify and produce valid results. Therefore, human expertise remains crucial for ensuring the accuracy and reliability of the simulation outputs.

AI-POWERED RESOURCE OPTIMIZATION FOR ENHANCED DECISION-MAKING PROCESSES

This study demonstrated that GPT-4o can determine the optimal allocation of resources to enhance simulated systems. Furthermore, the language model automatically generated and presented several viable simulation scenarios. By leveraging the capabilities of GPT-4o, the LLM can provide decision-makers with executable Python code that presents these scenarios and identifies the optimal resource allocation, thereby enabling more informed decision-making processes. Additionally, prompt engineering techniques can significantly improve the productivity of the decision-making process by enabling rapid modifications to text prompts, thus facilitating the exploration of various scenarios and their associated outcomes. These findings suggest that AI-driven resource allocation has substantial potential for improving the decision-making process.

CONCLUSIONS

Integrating NLP with DES has shown potential for automating the development and optimization of healthcare system models. In response to the first research question, this study successfully demonstrated that LLMs, such as GPT-4o, can generate DES models from natural language descriptions, thereby reducing the time and expertise required to model complex healthcare processes. The ability to automatically translate descriptive text into executable simulation codes allows for the rapid creation of models suitable for varying levels of complexity, making DES more accessible to healthcare professionals without specialized simulation knowledge. The study results also highlight integrating traditional simulation software with LLMs to expedite the simulation process.

For the second research question, applying NLP techniques in interpreting simulation results has proven to be effective in optimizing resource allocation. By analyzing the output of DES models, language models can generate actionable insights, such as identifying optimal staffing levels, to improve the efficiency of healthcare operations. This research demonstrates the potential of AI-driven systems to enhance decision-making processes in healthcare, providing a scalable and flexible approach to managing complex systems.

Future work can explore using Sora, an innovative framework for text-to-image and text-to-animation generation developed by OpenAI, to animate simulation models based on natural-language descriptions[32]. This emerging research area could provide a visual representation of simulation processes, further enhancing accessibility for non-technical users. Additionally, leveraging GPT models to create digital twins of healthcare facilities could allow for continuous monitoring, simulation, and optimization, thereby advancing the integration of AI in dynamic and real-time decision-making environments. Furthermore, this study highlights how traditional simulation software can integrate LLMs to expedite the simulation process. Combining the strengths of conventional simulation tools with AI-powered language models creates possibilities for faster, more efficient simulations in complex domains such as healthcare, reducing the need for manual intervention, and enabling quicker resolutions in decision-making. These future directions promise to expand the capabilities of current simulation systems, offering more sophisticated solutions to complex healthcare challenges.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript. There are no financial interests to disclose. We certify that the submission is original work and is not under review at any other publication.

DISCLOSURE

The authors did not receive any form of commercial support, either in the form of compensation or financial

assistance, for this case report. The authors have no financial interest in any of the products, devices, or drugs mentioned in this article.

ETHICAL APPROVAL

This study did not involve human participants, animal subjects, or any sensitive data requiring ethical approval. Since the research was limited to a comparative analysis of software tools, an ethical review was not applicable

DATA AVAILABILITY STATEMENT

The experiments were conducted in a reproducible manner and the source code is available in the GitHub repository: https://github.com/AGMiski/Modeling-Discrete-Event-Simulations-Using-Natural-Language-Processing-A-Healthcare-Application.

DISCLAIMER/PUBLISHER'S NOTE

The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

REFERENCES CITED

[1] Wallrath R, Franke MB. Integrating MILP, Discrete-Event Simulation, and Data-Driven Models for Distributed Flow Shop Scheduling Using Benders Cuts. Processes 2024, 12, 1772. https://doi.org/10.3390/pr12081772.

[2] Vázquez-Serrano JI, Peimbert-García RE, Cárdenas-Barrón LE. Discrete-Event Simulation Modeling in Healthcare: A Comprehensive Review. Int. J. Environ. Res. Public Health 2021, 18, 12262. https://doi.org/10.3390/ijerph182212262.

[3] Ortiz-Barrios M, Arias-Fonseca S, Ishizaka A, Barbati M, Avendaño-Collante B, Navarro-Jiménez E. Artificial Intelligence and Discrete-Event Simulation for Capacity Management of Intensive Care Units during the Covid-19 Pandemic: A Case Study. J. Bus. Res. 2023, 160, 113806. https://doi.org/10.1016/j.jbusres.2023.113806.

[4] Pradelli L, Eandi M, Povero M, Mayer K, Muscaritoli M, Heller AR, Fries-Schaffner E. Cost-Effectiveness of Omega-3 Fatty Acid Supplements in Parenteral Nutrition Therapy in Hospitals: A Discrete-event simulation Model. Clin. Nutr. 2014, 33, 785–792. https://doi.org/10.1016/j.clnu.2013.11.016.

[5] Lenin RB, Lowery CL, Hitt WC, Manning NA, Lowery P, Eswaran H. Optimizing Appointment Template and Number of Staff of an OB/GYN Clinic—Micro and Macro Simulation Analyses. BMC Health Serv. Res. 2015, 15, 1–17. https://doi.org/10.1186/s12913-015-1007-9.

[6] Sala F, Quarto M, D'urso G. Simulation Study of the Impact of COVID-19 Policies on the Efficiency of a Smart Clinic MRI Service. Healthcare 2022, 10, 619. https://doi.org/10.3390/healthcare10040619.

[7] Forbus JJ, Berleant D. Using Discrete-Event Simulation to Balance Staff Allocation and Patient Flow between Clinic and Surgery. Modelling 2023, 4, 567–584. https://doi.org/10.3390/modelling4040032.

[8] Jackson I, Jesus Saenz M, Ivanov D. From Natural Language to Simulations: Applying AI to Automate Simulation Modelling of Logistics Systems. Int. J. Prod. Res. 2024, 62, 1434–1457. https://doi.org/10.1080/00207543.2023.2276811.

[9] Alahmadi MD, Alshangiti M. Optimizing OCR Performance for Programming Videos: The Role of Image Super-Resolution and Large Language Models. Mathematics 2024, 12, 1036. https://doi.org/10.3390/math12071036.

[10] Otter DW, Medina JR, Kalita JK. A Survey of the Usages of Deep Learning for Natural Language Processing. IEEE Trans. Neural Netw. Learn. Syst. 2021, 32, 604–624. https://doi.org/10.1109/TNNLS.2020.2979670.

[11] Min B, Ross H, Sulem E, Veyseh APB, Nguyen TH, Sainz O, Agirre E, Heintz I, Roth D. Recent Advances in Natural Language Processing via Large Pre-Trained Language Models: A Survey. ACM Comput. Surv. 2023, 56, 1–40. https://doi.org/10.1145/3605943.

[12] Hu L, Liu Z, Zhao Z, Hou L, Nie L, Li J. A Survey of Knowledge Enhanced Pre-Trained Language Models. IEEE Trans. Knowl. Data Eng. 2024, 36, 1413–1430. https://doi.org/10.1109/TKDE.2023.3310002.

[13] Brito KDS, Filho RLCS, Adeodato PJL. A Systematic Review of Predicting Elections Based on Social Media Data: Research Challenges and Future Directions. IEEE Trans. Comput. Soc. Syst. 2021, 8, 819–843. https://doi.org/10.1109/TCSS.2021.3063660.

[14] Kastrati Z, Dalipi F, Imran AS, Nuci KP, Wani MA. Sentiment Analysis of Students' Feedback with Nlp and Deep Learning: A Systematic Mapping Study. Appl. Sci. 2021, 11, 3986. https://doi.org/10.3390/app11093986.

[15] Lin WY. Prototyping a Chatbot for Site Managers Using Building Information Modeling (BIM) and Natural Language Understanding (NLU) Techniques. Sensors 2023, 23, 2942. https://doi.org/10.3390/s23062942.

[16] García-Méndez S, Fernández-Gavilanes M, Costa-Montenegro E, Juncal-Martínez J, Javier González-Castaño F. A Library for Automatic Natural Language Generation of Spanish Texts. Expert Syst. Appl. 2019, 120, 372–386. https://doi.org/10.1016/j.eswa.2018.11.036.

[17] Mulla N, Gharpure P. Automatic Question Generation: A Review of Methodologies, Datasets, Evaluation Metrics, and Applications. Prog. Artif. Intell. 2023, 12, 1–32.

[18] Lin Y, Ruan T, Liu J, Wang H. A Survey on Neural Data-to-Text Generation. IEEE Trans. Knowl. Data Eng. 2024, 36, 1431–1449. https://doi.org/10.1109/TKDE.2023.3304385.

[19] Gartner D, Padman R. Machine Learning for Healthcare Behavioural OR: Addressing Waiting Time Perceptions in Emergency Care. J. Oper. Res. Soc. 2020, 71, 1087–1101. https://doi.org/10.1080/01605682.2019.1571005.

[20] Hosseini-Shokouh SM, Mohammadi K, Yaghoubi M. Optimization of Service Process in Emergency Department Using Discrete-event simulation and Machine Learning Algorithm. Arch. Acad. Emerg. Med. 2022, 10, e44. https://doi.org/10.22037/aaem.v10i1.1545.

[21] Olave-Rojas D, Nickel S. Modeling a Pre-Hospital Emergency Medical Service Using Hybrid Simulation and a Machine Learning Approach. Simul. Model. Pract. Theory 2021, 109, 102302. https://doi.org/10.1016/j.simpat.2021.102302.

[22] Atalan A, Şahin H, Atalan YA. Integration of Machine Learning Algorithms and Discrete-Event Simulation for the Cost of Healthcare Resources. Healthcare 2022, 10, 1920. https://doi.org/10.3390/healthcare10101920.

[23] Bengio Y, Ducharme R, Vincent P, Jauvin C, Ca, JU, Kandola J, Hofmann T, Poggio T, Shawe-Taylor J. A Neural Probabilistic Language Model. J. Mach. Learn. Res. 2003, 1, 1137–1155. https://doi.org/10.1162/153244303322533223.

[24] Vaswani A, Shazeer N, Parmar N, Uszkoreit J, Jones L, Gomez AN, Kaiser L, Polosukhin I. Attention Is All You Need. Adv Neural Inf Process Syst 2017.

[25] Achiam J, Adler S, Agarwal S, Ahmad L, Akkaya I, Aleman FL, Almeida D, Altenschmidt J, Altman S, et al. GPT-4 Technical Report. arXiv preprint arXiv:2303.08774 2023.

[26] Alahmadi MD, Alshangiti M, Alsubhi J. SCC-GPT: Source Code Classification Based on Generative Pre-Trained Transformers. Mathematics 2024, 12, 2128. https://doi.org/10.3390/math12132128.

[27] Yenduri G, Ramalingam M, Selvi GC, Supriya Y, Srivastava G, Maddikunta PKR, Raj GD, Jhaveri RH, Prabadevi B, Wang W, et al. GPT (Generative Pre-Trained Transformer)—A Comprehensive Review on Enabling Technologies, Potential Applications, Emerging Challenges, and Future Directions. IEEE Access 2024, 12, 54608–54649. https://doi.org/10.1109/ACCESS.2024.3389497.

[28] Lo, LS. The CLEAR Path: A Framework for Enhancing Information Literacy through Prompt Engineering. J. Acad. Librariansh. 2023, 49, 102720. https://doi.org/10.1016/j.acalib.2023.102720.

[29] Lee M. A Mathematical Investigation of Hallucination and Creativity in GPT Models. Mathematics 2023, 11, 2320. https://doi.org/10.3390/math11102320.

[30] Zhao Q, Li J, Liu J, Kang Z, Zhou Z. Is Word Order Considered by Foundation Models? A Comparative Task-Oriented Analysis. Expert Syst. Appl. 2024, 241, 122700. https://doi.org/10.1016/j.eswa.2023.122700.

[31] Lindemann NF. Chatbots, Search Engines, and the Sealing of Knowledges. AI Soc. 2024. https://doi.org/10.1007/s00146-024-01944-w.

[32] Brooks T, Hellsten J, Aittala M, Wang T-C, Aila T, Lehtinen J, Liu M-Y, Efros AA, Karras T. Generating Long Videos of Dynamic Scenes. Adv Neural Inf Process Syst 2022, 35, 31769--31781

REVIEW ARTICLE

Prevalence, Clinical Features, and Management of Hydatid Disease in Saudi Arabia: Systematic Review

Faten A. Al Braikan, MSc, PhD

Department of Clinical Microbiology and Immunology, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia

Correspondence
Dr. Faten A. Al Braikan
Department of Clinical Microbiology and Immunology, Faculty of Medicine
King Abdulaziz University
P.O. Box 80205, Jeddah 21589
Kingdom of Saudi Arabia
e-M: falbraikan@kau.edu.sa

Submission: 02 Jun. 2024
Accepted: 15 Nov. 2024

Citation
Al Braikan FA. Prevalence, clinical features, and management of hydatid disease in Saudi Arabia: Systematic review. JKAU Med Sci 2024; 31(2): 49-61. DOI: 10.4197/Med.31-2.5.

Abstract

Hydatidosis, caused by Echinococcus granulosus, is an endemic parasitic disease worldwide. The most frequent anatomic locations are the liver and lung. This systematic review examined the prevalence, clinical features, diagnosis, and management of hydatid disease in Saudi Arabia. Six databases (PubMed, Medline, Ovid, Scopus, Web of Science, and Cochrane) were searched using the keywords "Hydatid and Saudi Arabia" in the title and abstract. All papers investigating the hydatid diseases in Saudi Arabia were included in the systematic review. Eight articles were considered suitable for the systematic review of 82 papers that were extracted through the database search. The studies enrolled 835 patients, of whom 440 cases were diagnosed with hydatid disease. The prevalence of hydatid disease among patients with liver diseases ranged between 3.9 and 5.6% during the period from 1978 to 2014. In addition, the liver was the most reported infected organ with hydatid diseases, followed by the lungs among patients. Some studies diagnosed hydatid disease among asymptomatic patients, while others reported gastrointestinal, respiratory, and musculoskeletal symptoms. The most commonly reported diagnostic methods were indirect hemagglutination (IHA), ultrasonography, and Computed Tomography (CT) scans for locating cysts. A combined approach of surgical interventions such as endocystectomy and cystoperiocystectomy with medical therapy has shown greater effectiveness despite some associated complications like anaphylaxis, mild hypernatremia, biliary leakage, and bronchopleural fistula. Improving treatment outcomes and minimizing complications requires a multidisciplinary approach that combines surgical expertise with medical therapy, increased public awareness, and early diagnosis promotion.

Keywords

Prevalence, Diagnosis, Hydatid, Echinococcosis, Saudi Arabia

INTRODUCTION

Hydatid disease or cystic echinococcosis is a parasitic infectious disease that can be transmitted from animals to humans caused by the larval stage of the tapeworm Echinococcus[1]. Human infection with the parasite can occur through the consumption of food or water contaminated with its eggs or through direct contact with infected dog feces[2]. Four species of Echinococcus may be associated with human infection: *Echinococcus granulosus*, *Echinococcus multilocularis*, *Echinococcus vogeli*, and *Echinococcus oligoarthritis*. The most prevalent of these is tapeworm *E. granulosus*, which is responsible for cystic echinococcosis globally[3,4]. Other species of Echinococcus cause uncommon medical conditions. For instance, *E. multilocularis*, the juvenile stage of the fox tapeworm, leads to alveolar Echinococcus[5]. This condition generally appears as a lesion that occupies space in the liver and spreads to other organs. *E. vogeli* and *E. oligoarthritis* are the species responsible for polycystic echinococcosis, which presents as multiple cysts on almost any organ[6].

Hydatid disease is an international public health problem. It commonly affects developing countries, South America, West China, the Middle East, and North Africa (MENA), and other countries. The overall prevalence of the disease is underestimated due to low surveillance studies conducted in all prevalent regions. It was estimated that Hydatid disease has a global incidence rate of 1–200 per 100,000 yearly [7]. Furthermore, the number of cases affected by hydatid disease in the MENA region in 1990 was 134980 and increased to 207368 in 2019[8].

Hydatid disease tends to affect the liver (50%–77% of cases) due to its bowel venous drainage system. However, it can involve any visceral organ in the body, including the lung (15%–47%), spleen (0.5%–8%), kidney (2%–4%), and other organs[9,10]. Despite the unusual involvement of organs other than the liver and lung, it can cause substantial morbidity and mortality[11-13].

The disease could present with a range of symptoms that are not specific to the disease itself. The clinical manifestation of patients with hydatid disease depends on the organ affected and the size of the cyst, as well as the interaction between the cysts and the structure of the organ due to obstruction of blood/lymphatic flow, rupture, or subsequent bacterial infection. The infection may remain asymptomatic in earlier stages when the cyst is small (<10 cm) and may remain for many years; however, serious complications can occur, including peritonitis, anaphylactic shock, and multiorgan failure. Accordingly, disease management is crucial to reduce the possible complications[14,15]. In addition, secondary cyst infections are attributed to cystic-biliary communication. These infections are considered the most common risk factors for elevated intracystic pressure, erosion of adjacent structures by an enlarging cyst, and complications developing[15-17].

Abnormal laboratory findings, including thrombocytopenia, leukopenia, and elevated liver function, are observed in hydatid disease; however, these are nonspecific and not diagnostic. Several approaches are used for diagnosing the disease, including serological tests, radiological evaluation, and histopathological and cytological examinations[18,19]. The combination of imaging and serology investigations usually enhances diagnosis[20]. Regarding radiological evaluation, ultrasound imaging is a highly sensitive and efficient way to detect liver lesions[21]. Moreover, Computed Tomography (CT) scans and Magnetic resonance imaging (MRIs) are more effective in detecting and characterizing hydatid disease with greater sensitivity and specificity[22]. CT scans can quickly and accurately diagnose cyst ruptures, providing the exact location and type and allowing prompt surgical intervention in emergencies[23]. In terms of serological tests, Casoni skin and the indirect hemagglutination (IHA) methods are the most common approaches to detect the disease. The Casoni skin test involves injecting hydatid cyst fluid into the skin, while the IHA is a serum antibody test that is highly sensitive to the disease[24].

Treatment of hydatid cysts is attributed to their size, location, clinical signs and symptoms, and patient characteristics. Management usually includes antiparasitic treatment, surgical resection of the cyst, or percutaneous puncture, aspiration, injection, and respiration (PAIR), depending on the World Health Organization (WHO) diagnostic classification[25]. Smaller, uncomplicated liver cysts less than 5 cm in size can be effectively treated with albendazole alone or in combination with praziquantel. However, surgery is recommended for cysts larger than 10 cm and for cysts at risk of rupture or complications. In particular, the total cystopericystectomy technique is preferred as it has lower risks of postoperative complications[26]. Additionally, Endocystectomy is used for hepatic cystic

echinococcosis management, which is the conservative and feasible surgical approach[27].

Limited studies were conducted in Saudi Arabia to describe hydatid disease infection, its epidemiology, clinical presentation, diagnosis, and management to raise awareness of the problem and improve early diagnosis and management of the disease. Thus, we conducted this systematic review of the existing literature to provide an overview of the clinical features, management, and outcomes of hydatid disease in Saudi Arabia.

MATERIAL AND METHODS

This systematic review complied with established criteria (Preferred Reporting Items for Systematic Reviews and Meta-Analyses, PRISMA)[28].

SEARCH STRATEGY

The systematic review was conducted through a literature search of PubMed, Medline, Ovid, Scopus, web of Science, and Cochrane databases using the keywords "Hydatid AND Saudi Arabia" in the abstract and title. The author screened studies examining hydatid disease in Saudi Arabia to select studies that matched the inclusion and exclusion criteria. Furthermore, key data points were retrieved from the final record of the included research.

INCLUSION AND EXCLUSION CRITERIA

The systematic review included all papers investigating hydatid diseases in Saudi Arabia: prevalence, involved organs, investigation, management, and disease outcome. Studies were selected based on the following criteria: published in English, involving clinical samples, and full-text availability. We excluded duplicated papers, published studies in languages other than English**,** narrative reviews, case reports, case series, studies with insufficient data or findings, studies with irrelevant findings, studies that did not include clinical samples and studies for which full text was unavailable.

SCREENING AND DATA EXTRACTION

A reference manager was used to check the output of the search technique for duplication. The titles and abstracts of the relevant studies were first screened. Afterward, relevant full-text papers were examined for inclusion criteria. An independent author independently extracted data from a Microsoft Excel spreadsheet. The data included authors, year of publication, study design and period, objective, population characteristics, and study outcomes.

STRATEGY FOR DATA SYNTHESIS

A summary table was created using data from relevant studies to provide a qualitative interpretation of the findings and study components.

RISK OF BIAS ASSESSMENT

Out of 82 extracted studies, eight met our inclusion criteria and were assessed for risk of bias using the ROBINs-1 tool[29] among non-randomized studies of interventions (NRSI). This tool was used to evaluate the quality of studies included, assessing aspects such as confounding, selection bias, measurement of outcomes, and intervention classification. The studies' risk of bias was categorized as low, moderate, serious, or critical based on predefined criteria. The overall risk of bias was reached using signaling questions. The risk of bias revealed the overall quality of the included studies.

RESULTS

OVERVIEW OF THE INCLUDED STUDIES

Out of 82 papers were extracted from six databases (PubMed, Medline, Ovid, Scopus, Cochrane, and Web of Science) search. Of these, 28 were omitted as duplicates. Regarding the remaining 54 articles, 44 were excluded because they did not match the inclusion criteria: 3 were review articles, 19 did not include clinical samples, 16 were case reports, 2 were case series, and 4 were not full-access papers. Following screening and assessment, 2 articles were excluded because they did not match the study's objective. Eight articles were considered suitable for the systematic review (Figure 1).

The included papers were published in different hospital settings in Saudi Arabia, mainly in two cities (Riyadh and Jeddah). The studies enrolled 835 patients, of whom 440 cases were diagnosed with hydatid disease. The included studies were published between 1983 and 2016 (Table 1). The study design varied among the included studies; one of the papers utilized a case-control study design, six relied on retrospective analysis, and one was prospective studies. The studies

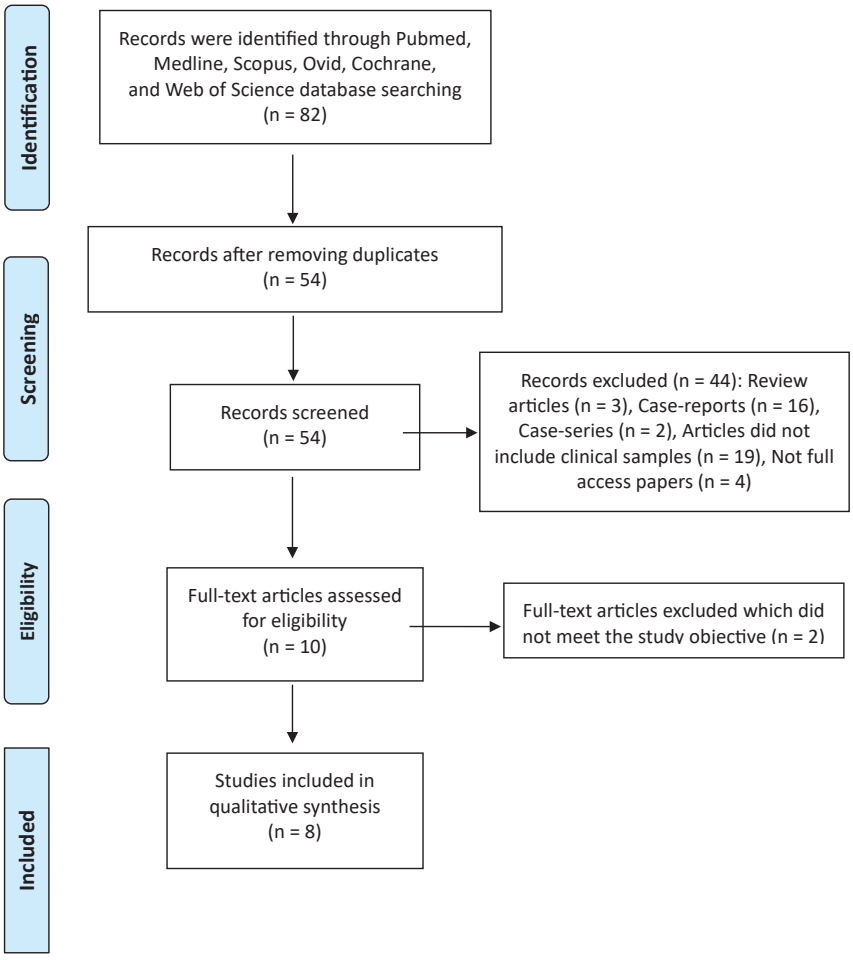


Figure 1. **Flow diagram of study selection for the systematic review**

Table 1. **Characteristics of the included studies.**

Study Reference Number	Study Design (Period)	Study Objective	Population Characteristic	Results
30	Retrospective (from March 1978 to September 1980)	To identify clinicopathological patients with liver disease	**Number:** 124 with liver disease. Out of 7 patients, they were diagnosed with hydatid disease. **Gender:** 4 males and 3 females with hydatid disease. **Age:** ranged from 17 to 50 years.	The prevalence of hydatid liver disease among patients with liver diseases who had liver biopsy performed was 5.6%. **Laboratory findings:** All patients had a normal level of bilirubin. SGOT levels ranged from 6 to 54 (i.u.), while SGPT levels ranged from 4 to 38 (i.u.). Alkaline phosphatase levels ranged from 18 to 94 (i.u.). Alpha-fetoprotein was tested among one patient (1/20000 ng ml-1). Eosinophilia was detected in four patients.

Table 1. Characteristics of the included studies.–Continued

Study Reference Number	Study Design (Period)	Study Objective	Population Characteristic	Results
31	Case-control study	Serodiagnosis of human hydatid disease	**Number:** Control group: 120 male blood donors + 114 patients (adult females and children) with no history of hydatid disease. Test group: 52 suspected hydatid patients.	**Diagnosis:** Out of 52 patients who were suspected to have human hydatid disease, 30 cases tested positive through serodiagnosis by IHA test. Among 20 cases of hydatid liver cysts, three were confirmed through ultrasonography, resulting in an 80% positivity rate. Among patients suspected of having hydatid disease (hepatomegaly, splenomegaly, or hepatosplenomegaly) and exhibiting symptoms, 50% had IHA titers of 1:128 or higher. The serological results of this control group showed that 70% had no hydatid IHA antibodies, while 25% had antibody titers of 1:4 to 1:8.
32	Retrospective study (September 1984 to August 1986	To describe the management of liver hydatidosis.	**Number:** 68 patients with hydatid disease involving various organs (liver, lung, and others (brain, kidney, spleen, abdominal wall) at Riyadh Central Hospital. **Gender:** Male: 15 Females: 27 **Age:** from 8 to more than 60 years.	**Diagnosis:** 42 patients were diagnosed with liver cysts. The right lobe was the site with the most involvement (71%). Ultrasonography and Casoni's intradermal test were helpful in diagnosing cysts. CT was used to better localize cysts and plan suitable surgical procedures. **Organ involved:** •Liver: Liver alone (37 patients), liver and spleen (2 patients), liver and lung (2 patients), Liver, spleen, and kidney (1 patient). •Lung: Lung alone (20 patients), lung and spleen (1 patient), and lung and breast (1 patient). •Other organs: Brain (1 patient), kidney (1 patient), spleen (1 patient) and abdominal wall (1 patient). **Symptoms:** Abdominal pain (29 patients), abdominal mass (17 patients), jaundice (5 patients), and asymptomatic (4 patients). **Laboratory findings:** Leukocytosis (> 12,000/mm3): 8 patients (17%). Eosinophilia (>8%): 14 patients (31.1%). Serum bilirubin (> 2 mg/dl): 5 (11.1%). Alkaline phosphatase (> 200 IU/dl): 10 patients (22.2%). Positive Casoni's test: 26 of 30 patients (87%). Positive IHA test: 20 of 28 patients (71%). **Scan findings:** -15 cases experienced plain abdominal and chest roentgenograms. •A raised dome of the diaphragm was detected in nine cases. •Soft tissue mass was detected in three cases. • Calcification was detected in three cases. - Ultrasound examination identified the cystic mass in all cases. -An isotope liver scan was performed among 15 patients, and all had the filling defect due to the cyst. -A CT scan was needed in 20 cases for better cyst localization.

Table 1. Characteristics of the included studies.–Continued

Study Reference Number	Study Design (Period)	Study Objective	Population Characteristic	Results
				- An ERCP was performed on four patients with high serum bilirubin. - The remaining patient with high bilirubin had PTC. **Management:** Out of 33 patients underwent surgery. Endocystectomy with tube drainage was performed among 23 patients, and five patients underwent total cystoperiocystectomy. One case received medical treatment. **Outcome:** No complications were noted in these patients. One patient died of anaphylaxis following percutaneous transhepatic cholangiography. A 20% hypertonic saline solution was used as a scolicidal agent in this series, and mild hypernatremia was noted among some patients.
33	Descriptive study (between 1988 and 1997)	To investigate the epidemiology of hydatid disease	**Number:** 67 patients diagnosed with *E. granulosus* infection. **Gender:** Male: 53.7%, Females: 46.3% **Age:** mean: 39.2 (SD:18.2) years.	**Diagnosis:** All patients were diagnosed using serology, including IHA, Casoni skin test, radiography, ultrasound, and CT. **Symptoms:** Half of the patients had gastrointestinal symptoms, 49.3%. Respiratory (32.8%), genitourinary (11.9%), and musculoskeletal systems were also involved (3%). Approximately 3% of the patients were asymptomatic. Out of 40 patients had hepatic involvement, of which four were considered secondary, and the majority had cysts in the right lobe. One patient complained of back pain. **Cyst location:** The cyst was found in the spleen, the tail of the pancreas, and the extensor muscles of the back and the lower limbs. **Treatment:** 56.7% of patients received surgical treatment, 20.9% had surgical and medical therapy, and 22.4% had albendazole. **Outcome:** Out of 97% of the patients survived, but 20% had a disease recurrence. Two patients died with a case fatality rate of 3%. One patient (a 66-year-old female) died two weeks after the operation for hydatid disease of the liver and bronchus. The other patient was an 85-year-old who had had surgery five years earlier and died of unknown causes.
34	Retrospective study (from 1983-1996)	To assess the medical records of patients with histopathologic diagnosis of hepatic granuloma	**Number:** 116 patients **Gender:** Male: 77. Females: 39 **Age:** from 18 to 65 years.	The prevalence of hydatid disease among patients who had hepatic granulomas identified by liver biopsy was 4.3%. **Symptoms:** Anorexia and abdominal pain.

Table 1. Characteristics of the included studies.–Continued

Study Reference Number	Study Design (Period)	Study Objective	Population Characteristic	Results
35	Observational study (December 1999 to December 2004)	To describe the presentation, diagnosis, and management of patients suffering from Echinococcosis.	**Number:** 117 patients with echinococcosis. **Gender:** Male: 63.24%, Females: 36.76% **Age:** mean: 40.9 (SD: 20.7) years.	**Clinical presentation:** Out of 29 patients (24.8%), complications related to Echinococcosis were present. **Symptoms:** The most typical symptoms were pain in the right upper quadrant (51 cases, 43.6%), followed by cough ± sputum (22 cases, 18.8%). Pain in the upper abdomen was detected in 21 cases, or 17.9%. Nine patients were presented with jaundice ± cholangitis. A few cases were presented with fever and Lump L Thigh (3 and 1 case, respectively). **Diagnosis:** **Laboratory findings:** The IHA test gave a sensitivity of 78.6. The initial results of the hematological profile indicated a mean hemoglobin level of 12.7 (SD:17.4) g/dl and a mean white blood cell count of 9.3 (SD:4.9) /cc. **Scans:** All cysts were visualized using ultrasound scans (cases with extrapulmonary Echinococcosis), chest X-rays, and CT scans (used in selected cases). **Management:** Endocystectomy was the most frequent procedure in both the liver and lung. Albendazole was the most frequently used drug among 78 cases (68.4%) at a dose of 15 mg/kg in two divided doses. Mebendazole was used among the rest of the patients at a 50 mg/kg dose in three divided doses. Both these drugs were efficacious. However, Albendazole was more efficacious than Mebendazole. **Outcome:** Eight patients experienced biliary leakage, while three suffered from bronchopleural fistula. Most patients with these conditions could recover with conservative treatment, with only two of the biliary leakage cases and one of the bronchopleural fistula cases requiring surgery. Additionally, 15 patients experienced complications related to infections. No recurrences were recorded during the follow-up period; all patients who underwent surgery also received medical treatment. However, multiple factors prevented 25 patients (21.4%) from undergoing operative treatment and were treated with medical care. The mean hospital stay was 14.4 days (2–187 days). Patients receiving surgical treatment were discharged earlier, with a mean stay of 11.9 days, while those receiving medical treatment alone were generally sicker and tended to stay longer, with a mean of 23.6 days.
36	Retrospective study (2006-2014)	To measure morbidity and	**Number:** 77 patients underwent hepatectomy	The prevalence of hydatid cysts among patients undergoing liver resection was 3.9% as a benign indication.

Table 1. Characteristics of the included studies.–Continued

Study Reference Number	Study Design (Period)	Study Objective	Population Characteristic	Results
		mortality and assess predictors of outcome after hepatectomy.	**Gender:** Male: 53.3%, Females: 46.8% **Age:** mean: 49.1 years.	
37	Prospective study, (1995 to 2005)	To identify risk factors associated with endobronchial rupture among patients with hydatid cysts.	**Number:** 32 patients undergoing thoracotomies for pulmonary hydatid cysts **Gender:** Male: 21 Females: 11 **Age:** mean: 32 (SD: 15) years.	**Diagnosis:** Chest radiography and CT were used to identify the cysts' precise location, size, and integrity. **Outcome:** 53.1% of the patients (17 in total) had ruptured cysts (group 1), while 15 patients had intact cysts (group 2). Most cysts (68.7%) were found in the right lung. Out of 21 (65.6%) cysts were identified in the lower lobes, 6 (18.7%) in the upper lobes, and 5 (15.6%) in the lingual and middle lobes. The fistula diameter was the only significant risk factor associated with cyst rupture.

IHA = Indirect hemagglutination; CT = Computerized tomography; ERCP = Endoscopic retrograde cholangiopancreatography; PTC = per-cutaneous transhepatic cholangiography; SD = Standard deviation.

included diverse patient populations within the age range of six months to 95 years, and most of them were patients with liver disease.

RISK OF BIAS ASSESSMENT OF THE INCLUDED STUDIES

Regarding the risk of bias assessment, Table 2 summarizes the studies that have been included in the hydatid disease pattern. Based on our assessments, the overall quality was between moderate and serious. The probable sources of bias were the presence of confounding, measurement of reported results, and classification of interventions that were mostly reported in an unclear way for a conclusive judgment.

DISCUSSION

Hydatid disease is an endemic chronic disease in certain areas that causes devastating acute complications[38,39]. Few clinical studies on hydatid diseases were carried out in Saudi Arabia. Therefore, this systematic review aims to investigate the prevalence, clinical features, diagnosis, management, and prognosis of hydatid disease in Saudi Arabia.

The prevalence of hydatid disease was reported only among patients with liver diseases. In the current systematic review, three studies discussed the prevalence of hydatid diseases among patients with liver diseases only. The prevalence of hydatid disease was 5.6% from 1978 to 1980 in Jeddah. However, in Riyadh, the rate decreased to 4.3% from all liver biopsy specimens obtained over 14 years from 1983 to 1996. During the period from 2006 to 2014, the rate was 3.9%[30,34,36]. These findings suggest a positive development in the management and control of hydatid diseases among patients with liver conditions. The decreasing prevalence may be attributed to various factors, such as advancements in medical technology, improved diagnostic techniques, and enhanced public health measures. WHO has estimated that the rate of human infection exceeds 50 per 100,000 individuals each year in areas where echinococcosis is endemic. High prevalence is noted in various regions of Argentina, Central Asia, and East Africa[40]. In our study, it is difficult to estimate the prevalence of hydatid disease as these data were extracted from symptomatic patients who underwent liver biopsy or resection. Hence, this underestimated the total number of hydatid diseases in Saudi Arabia. Future studies should strive to encompass a broader range of patient demographics and disease presentations in recent years to gain a more comprehensive understanding of the true prevalence of the disease and avoid prevalence influenced by changes in public health across years.

Table 2. Robvis traffic light plot pigure

Study ID	D1	D2	D3	D4	D5	D6	D7	Overall
Kassimi et al., 1983[30]	-	+	-	-	+	+	-	-
Hossain et al., 1985[31]	-	+	-	+	X	+	X	X
Al-Kraida et al., 1988[32]	-	+	-	+	+	-	-	-
Alam, 1999[33]	-	+	-	+	+	+	X	X
Al Mofleh et al., 2000[34]	-	-	+	-	-	+	+	-
Fahim, and Al Salamah, 2007[35]	-	+	+	+	+	+	-	-
Al-Alem et al., 2016[36]	-	+	-	+	+	+	-	-
Ashour et al., 2016[37]	-	-	+	-	+	-	-	-

Domains:
D1: Bias due to confounding
D2: Bias due to the selection of participants
D3: Bias in the classification of interventions
D4: Bias due to deviation from intended interventions
D5: Bias due to missing data
D6: Bias in measurements of outcomes
D7: Bias in measurement of reported results

Judgment
+ Low
- Moderate
X Serious

Moreover, future research should strive to encompass underrepresented regions, such as the southern and northern parts of Saudi Arabia.

In line with the literature, the organ most reported infected with hydatid diseases in our review was the liver, followed by the lungs. However, hydatid cysts could be seen in various other parts of the body. According to the included studies, the liver was the most reported infected site for hydatid diseases[30-32,34-36], whereas the right lobe was the most involved site among patients with hydatid liver disease[32-34]. In addition, the lung was the second affected organ, which was reported in three studies[32,33,35,37], where one study reported that most cysts were found in the right lung[37]. Rarely, cysts were also seen in other sites alone or with the liver and lung, such as the spleen, kidney, brain, breast, abdominal wall, the tail of the pancreas, peritoneal, and the extensor muscles of the back and the lower limbs[32,33].

There are variations in the clinical features of hydatid disease, which can make a presumptive

diagnosis difficult. From the included studies, four studies reported the symptoms of hydatid disease[32-35]. Hydatid disease can cause symptoms in various body systems, including the liver, gastrointestinal, respiratory, genito-urinary, and musculoskeletal. The reported signs and symptoms involve back and abdominal pain, even in the right upper quadrant of the abdomen and upper abdomen, anorexia, and hepatomegaly[32-35]. Other symptoms based on the site, such as cough, anorexia, fever, jaundice, incidental urticaria, anaphylactic reaction, and disease recurrence, may also occur[32,33,35]. Additionally, a lump on the left thigh and complications such as cholangitis, intrabiliary and intrabronchial rupture of the hydatid cyst, compression, and infection can be associated with hydatid disease[35,37]. However, three studies identified some asymptomatic patients with hydatid disease, and patients with primary pulmonary hydatidosis had concomitant asymptomatic hepatic cysts[32,33,35]. These symptoms are nonspecific, which may lead to ignorance, delay the diagnosis, and worsen the prognosis. The review outlines various symptoms of hydatid disease and emphasizes the need for a detailed analysis to differentiate these symptoms from other prevalent diseases in Saudi Arabia, like tuberculosis, to avoid misdiagnoses or delays in diagnosis. Future studies should focus on distinguishing these overlapping symptoms in clinical practice.

The laboratory findings discussed in the included studies revealed a wide range of variations in the biochemical parameters among patients with hydatid disease. For instance, bilirubin levels varied among the included studies. One study reported normal levels among patients with hydatid liver disease[30], and another study reported high levels among 11.1% of patients with the disease among different organs[32]. Moreover, one study reported the variation level of SGOT and SGPT; whereas the SGOT levels ranged from 6 to 54 i.u, SGPT levels ranged from 4 to 38 i.u[30]. Two studies reported alkaline phosphate levels, one of which reported variation from 18 to 94 i.u[30], and the other found that 22.2% had abnormal levels[32]. One study tested alpha-fetoprotein among one patient with hydatid disease with a result of 1/20000 ng ml-1[30]. Moreover, leukocytosis was detected in 17% of patients with hydatid disease[32]. Another study detected a high mean (Standard deviation [SD]) of leukocytes (9.3 (4.9)) /cc[35]. Additionally, two studies indicated eosinophilia among patients with hydatid disease, with prevalence rates of 5.8% and 31.1%[30,32]. Another study among patients with echinococcosis reported a mean (SD) hemoglobin level of 12.7 (17.4) g/dl[35]. The variations in the findings could be attributed to factors such as the stage of the disease, the involvement of different organs, and the individual patient's response to the infection.

Regarding serodiagnosis, four studies used the IHA test, which was practical for diagnosis because of its high sensitivity (50%, 71%, 78.6, and 100%) in diagnosing the hydatid disease[31-33,35]. One study reported that 50% of suspected hydatid disease patients had IHA titers of 1:128 or higher[31]. The increased IHA antibody production against parasite-associated antigens can lead to immunopathological lesions[41]. Additionally, two studies used Casoni's intradermal test (16.4% & 87%). Despite it concluded that it was helpful in diagnosis[32,33]. In accordance with previous studies, Casoni's test showed a varied accuracy (50 to 90%)[42-44]. Performing both the Casoni's and IHA tests in suspected hydatidosis patients enhances the diagnostic accuracy of the disease. In this review, the sensitivity and specificity of diagnostic methods for hydatid disease in various healthcare settings in Saudi Arabia have not been critically analyzed. Therefore, it's important to assess the accessibility of these diagnostic tools in all healthcare facilities and compare the techniques used to global standards for improved accuracy.

Regarding scan findings, four studies used ultrasonography, which effectively diagnosed hydatid diseases[31-33,35]. In addition, four studies used CT[32,33,35,37], and three used chest radiography[33,35,37] to identify the cysts' precise location, size, and integrity. Moreover, a study among patients with pulmonary disease used chest X-rays as a screening tool for the disease[35]. These findings underscore the significance of utilizing a combination of serodiagnostic tests and imaging techniques for the diagnosis of hydatid disease.

Surgery is currently the most effective treatment for removing *E. granulosus* cysts and achieving a complete cure, with a success rate of up to 90% in patients[45]. However, it may not be practical for some patients whose cysts are located in risky positions, multiple sites, or different organs, or who are at high risk or have inadequate surgical facilities[46]. In the present systematic review, four studies used surgery techniques to manage hydatid disease, such as endocystectomy[32,35] and cystoperiocystectomy[32], and two studies did not mention the surgery type used[33,37]. However, post-surgery complications were reported in

the studies, such as anaphylaxis, hypernatremia, biliary leakage, and bronchopleural fistula, which can occur in patients with hydatid disease[32,33,35,37].

Additionally, the included studies reported hydatid management through surgical and/or medical therapy such as mebendazole[35] or medical therapy only as albendazole[33,35] or antihelminthic[35], which effectively managed hydatid diseases. One study revealed that Albendazole was more effective than mebendazole[35]. On the other hand, it was found that patients who underwent surgical treatment tended to have shorter hospital stays than those who received only medical treatment[35]. The review covers surgical and medical management techniques but lacks a critical evaluation of their effectiveness and cost-benefit analysis. Future research should further explore the advantages of surgery over medical treatments, such as albendazole, in different clinical contexts. Additionally, a more comprehensive examination of complications and strategies for prevention would be valuable. Analyzing the long-term outcomes for patients with hydatid disease, including recurrence rates after treatment and potential chronic complications, would significantly enhance the review, as well as understanding the long-term socioeconomic impact of the disease, particularly for those in rural communities or with limited access to healthcare.

Considering that hydatid disease is zoonotic, it would be helpful to enhance the existing public health measures in Saudi Arabia aimed at controlling the disease. These measures could involve deworming livestock, enhancing sanitation, and educating at-risk populations about preventive practices. Furthermore, it would be beneficial to investigate the potential impact of veterinary and agricultural policies in reducing transmission, such as enforcing stricter hygiene standards in slaughterhouses and promoting responsible pet ownership. This could provide more practical insights[45]. Therefore, collaboration between veterinarians and public health workers is crucial for effectively controlling hydatidosis[47]. Moreover, increasing public awareness will improve treatment outcomes and minimize complications of hydatid disease in Saudi Arabia.

One limitation of this study is that the included studies were limited to a specific geographical area. The majority were conducted in Riyadh, and only one study was carried out in Jeddah. Therefore, the findings may not represent the true information about hydatid diseases in Saudi Arabia.

CONCLUSION

The systematic review highlights the presence of hydatid disease in Saudi Arabia. However, the prevalence cannot be truly estimated. The symptoms of hydatid disease were mainly in the gastrointestinal, respiratory, and musculoskeletal systems based on the site and size. The most common diagnostics tests were serological tests, such as IHA and Casoni's test, and imaging, such as ultrasonography and CT, for cyst locations. Endocystectomy and cystoperiocystectomy were effectively used to manage hydatid disease, where surgeries combined with medical therapy could be more effective. However, complications such as anaphylaxis, mild hypernatremia, biliary leakage, and bronchopleural fistula were reported. Increasing public awareness, promoting early diagnosis, and adopting a multidisciplinary approach combining surgical expertise with medical therapy are crucial to improving treatment outcomes and minimizing complications of hydatid disease in Saudi Arabia.

CONFLICT OF INTEREST

The author declared that there is no conflict of interest that is related to this study and this article.

DISCLOSURE

The author did not receive any form of commercial support, including compensation or financial assistance, for this case report. Additionally, the author has no financial interest in any of the products, devices, or drugs mentioned in this article.

ETHICAL APPROVAL

Not applicable.

REFERENCES CITED

[1] Eckert J, and Thompson RC. Historical Aspects of Echinococcosis. Adv Parasitol. 2017;95:1-64. doi:10.1016/bs.apar.2016.07.003

[2] McManus DP, Zhang W, Li J, and Bartley PB. Echinococcosis. Lancet. 2003;362(9392):1295-1304. doi:10.1016/S0140-6736(03)14573-4

[3] Polat P, Kantarci M, Alper F, Suma S, Koruyucu MB, and Okur A. Hydatid disease from head to toe. Radiographics. 2003;23(2):475-537. doi:10.1148/rg.232025704

[4] Torgerson PR, and Budke CM. Echinococcosis--an international public health challenge. Res Vet Sci. 2003;74(3):191-202. doi:10.1016/s0034-5288(03)00006-7

[5] Kern P, Wen H, Sato N, et al. WHO classification of alveolar echinococcosis: principles and application. Parasitol Int. 2006;55 Suppl:S283-S287. doi:10.1016/j.parint.2005.11.041

[6] Meneghelli UG, Martinelli AL, Llorach Velludo MA, Bellucci AD, Magro JE, and Barbo ML. Polycystic hydatid disease (Echinococcus vogeli). Clinical, laboratory and morphological findings in nine Brazilian patients. J Hepatol. 1992;14(2-3):203-210. doi:10.1016/0168-8278(92)90159-m

[7] Nunnari G, Pinzone MR, Gruttadauria S, et al. Hepatic echinococcosis: clinical and therapeutic aspects. World J Gastroenterol. 2012;18(13):1448-1458. doi:10.3748/wjg.v18.i13.1448

[8] Rahim F, Qasim NH, Zhumagaliuly A, and Dzzhusupov K. Human Cystic Echinococcosis in The Populations of MENA Countries, With A Focus on The United Arab Emirates, From 1990 To 2019: From Genetic Epidemiology of Rare Disease to Systematic Analysis for the Global Burden of Disease Study 2019. 2023 doi:10.21203/rs.3.rs-3190738/v1

[9] Akbulut S. Parietal complication of the hydatid disease: Comprehensive literature review. Medicine (Baltimore). 2018;97(21):e10671. doi:10.1097/MD.0000000000010671

[10] Fadel SA, Asmar K, Faraj W, Khalife M, Haddad M, and El-Merhi F. Clinical review of liver hydatid disease and its unusual presentations in developing countries. Abdom Radiol (NY). 2019;44(4):1331-1339. doi:10.1007/s00261-018-1794-7

[11] Ben-Hamda K, Maatouk F, Ben-Farhat M, et al. Eighteen-year experience with echinococcosus of the heart: clinical and echocardiographic features in 14 patients. Int J Cardiol. 2003;91(2-3):145-151. doi:10.1016/s0167-5273(03)00032-9

[12] Göğüş C, Safak M, Baltaci S, and Türkölmez K. Isolated renal hydatidosis: experience with 20 cases. J Urol. 2003;169(1):186-189. doi:10.1016/S0022-5347(05)64064-5

[13] Nourbakhsh A, Vannemreddy P, Minagar A, Toledo EG, Palacios E, and Nanda A. Hydatid disease of the central nervous system: a review of literature with an emphasis on Latin American countries. Neurol Res. 2010;32(3):245-251. doi:10.1179/016164110X12644252260673

[14] Dadoukis J, Gamvros O, and Aletras H. Intrabiliary rupture of the hydatid cyst of the liver. World J Surg. 1984;8(5):786-790. doi:10.1007/BF01655782

[15] Eckert J, Deplazes P, Craig PS, Gemmell MA, Gottstein B, Heath D, Jenkins DJ, Kamiya M, and Lightowlers M. Echinococcosis in animals: clinical aspects, diagnosis and treatment. WHO/OIE Manual on echinococcosis in humans and animals: a public health problem of global concern. 2001:72-99. doi: 10.5555/20013100617

[16] Mandolkar S, B R, PL A, GT S. Cystocutaneous fistula of the left lobe of liver: An extremely rare presentation of hydatid liver cyst. International Surgery Journal. 2015;2(1):109. doi:10.5455/2349-2902.isj20150224

[17] Kjossev KT, and Teodosiev IL. Cutaneous fistula of liver echinococcal cyst previously misdiagnosed as fistulizated rib osteomyelitis. Trop Parasitol. 2013;3(2):161-165. doi:10.4103/2229-5070.122150

[18] Kammerer WS, and Schantz PM. Echinococcal disease. Infect Dis Clin North Am. 1993;7(3):605-618.

[19] Bektas S, Erdogan NY, Sahin G, Kir G, and Adas G. Clinicopathological findings of hydatid cyst disease: a retrospective analysis. Ann Clin Pathol. 2016;4(3):1071. doi: 10.47739/2373-9282/1071

[20] BEKÇİ TT. Diagnosis and treatment of human hydatid disease. Eur. J. Gen. Med. 2012;9(12):15-20. doi:10.29333/ejgm/82498

[21] Safioleas M, Misiakos E, Manti C, Katsikas D, and Skalkeas G. Diagnostic evaluation and surgical management of hydatid disease of the liver. World J Surg. 1994;18(6):859-865. doi:10.1007/BF00299087

[22] Tüzün M, Altinörs N, Arda IS, and Hekimoğlu B. Cerebral hydatid disease CT and MR findings. Clin Imaging. 2002;26(5):353-357. doi:10.1016/s0899-7071(02)00449-7

[23] Alexiou K, Mitsos S, Fotopoulos A, et al. Complications of Hydatid Cysts of the Liver: Spiral Computed Tomography Findings. Gastroenterology Res. 2012;5(4):139-143. doi:10.4021/gr460e

[24] Tuazon AO, and Pasterkamp H. Hydatid disease of the lung (pulmonary hydatidosis). In: Chernick V, Boat TF, Kendig EL, editors. Kendig's disorders of the respiratory tract in children. Philadelphia (PA): WB Saunders. 1998;1050-7

[25] Brunetti E, Kern P, and Vuitton DA; Writing Panel for the WHO-IWGE. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. Acta Trop. 2010;114(1):1-16. doi:10.1016/j.actatropica.2009.11.001

[26] Ferrer Inaebnit E, Molina Romero FX, Segura Sampedro JJ, González Argenté X, and Morón Canis JM. A review of the diagnosis and management of liver hydatid cyst. Rev Esp Enferm Dig. 2022;114(1):35-41. doi:10.17235/reed.2021.7896/2021

[27] Al-Saeedi M, Ramouz A, Khajeh E, et al. Endocystectomy as a conservative surgical treatment for hepatic cystic echinococcosis: A systematic review with single-arm meta-analysis. PLoS Negl Trop Dis. 2021;15(5):e0009365. Published 2021 May 12. doi:10.1371/journal.pntd.0009365

[28] Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. Int J Surg. 2021;88:105906. doi:10.1016/j.ijsu.2021.105906

[29] Sterne JA, Hernán MA, Reeves BC, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of

interventions. BMJ. 2016;355:i4919. Published 2016 Oct 12. doi:10.1136/bmj.i4919

[30] Kassimi MA, Ali M, Zimmo SK, Khan MA, and Anees AM. Pattern of liver disease in the western region of Saudi Arabia. Ann Trop Med Parasitol. 1983;77(2):179-186. doi:10.1080/00034983.1983.11811695

[31] Hossain A, Bolbol AS, and Chowdhury MN. Serodiagnosis of human hydatid disease in Riyadh, Saudi Arabia. Ann Trop Med Parasitol. 1985;79(4):439-442. doi:10.1080/00034983.1985.11811942

[32] Al-Kraida A, Alam MK, Qazi S, Al-Qasabi QO, and Bashier AM. Hydatid disease of the liver in Riyadh. Ann. Saudi Med. 1988 Mar;8(2):117-21.

[33] Alam AA. Epidemiology of hydatid disease in Riyadh: A hospital-based study. Ann Saudi Med. 1999;19(5):450-452. doi:10.5144/0256-4947.1999.450

[34] Al Mofleh IA, Al Rashed RS, Ayoola EA, et al. Hepatic granulomas in an arab population: a retrospective study from a teaching hospital in Riyadh. Saudi J Gastroenterol. 2000;6(1):41-46.

[35] Fahim F, and Al Salamah SM. Cystic echinococcosis in Central Saudi Arabia: a 5-year experience. Turk J Gastroenterol. 2007;18(1):22-27.

[36] Al-Alem F, Mattar RE, Fadl OA, Alsharabi A, Al-Saif F, and Hassanain M. Morbidity and mortality and predictors of outcome following hepatectomy at a Saudi tertiary care center. Ann Saudi Med. 2016;36(6):414-421. doi:10.5144/0256-4947.2016.414

[37] Ashour MH, Hajjar WM, Ishaq M, et al. Pulmonary hydatid cysts: the naturally occurring models for rupture. Asian Cardiovasc Thorac Ann. 2016;24(7):670-675. doi:10.1177/0218492316658374

[38] Laajam MA, and Nouh MS. Hydatidosis: clinical significance and morbidity patterns in Saudi Arabia. East Afr Med J. 1991;68(1):57-63.

[39] Halezeroglu S, Celik M, Uysal A, Senol C, Keles M, Arman B. Giant hydatid cysts of the lung. J Thorac Cardiovasc Surg. 1997;113(4):712-717. doi:10.1016/S0022-5223(97)70228-9

[40] Lodhia J, Chugulu S, Sadiq A, Msuya D, and Mremi A. Giant isolated hydatid lung cyst: two case reports. J Med Case Rep. 2020 Oct 24;14(1):200. doi: 10.1186/s13256-020-02524-4.

[41] Warren KS. A functional classification of granulomatous inflammation. Ann N Y Acad Sci. 1976;278:7-18. doi:10.1111/j.1749-6632.1976.tb47011.x

[42] Kattan YB. Intrabiliary rupture of hydatid cyst of the liver. Br J Surg. 1975;62(11):885-890. doi:10.1002/bjs.1800621108

[43] Malaika SS, Attayeb A, Sulaimani S, and Reddy JJ (1981). Human echinococcosis in Saudi Arabia. Saudi Med. J. 1981;2:77-84. doi:10.5555/19832901052

[44] Langer JC, Rose DB, Keystone JS, Taylor BR, and Langer B. Diagnosis and management of hydatid disease of the liver. A 15-year North American experience. Ann Surg. 1984;199(4):412-417. doi:10.1097/00000658-198404000-00007

[45] Eckert J, Gemmell MA, Meslin FX, Pawlowski ZS. WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. Paris: World Organisation for Animal Health. 2002 Jan:20-72. Accessed 03 March 2024. https://www.who.int/publications/i/item/929044522X

[46] WHO Informal Working Group. Guidelines for the treatment of cystic and alveolar echinococcosis in humans. Bull WHO. 1996;74:231-42. Accessed 03 March 2024. https://cir.nii.ac.jp/crid/1573387451068892672

[47] Gessese AT. Review on Epidemiology and Public Health Significance of Hydatidosis. Vet Med Int. 2020 Dec 3;2020:8859116. doi: 10.1155/2020/8859116

REVIEW ARTICLE

Site-Directed Mutagenesis in Viral Glycoprotein and Its Role in Viral Diagnostics and Therapy

Basem A. Jawa[1], Msc, PhD, Khulud A. Alhazmi[2], Msc, PhD, Majed M. Shaikh[1], Marwan A. Albulushi[1], Mohammad M. Alkhozaee[1], Daee M. Almalki[1], Weam M. Filfilan[1], Afnan H. Falemban[1], Eman N. Alqurashi[1], Alaa A. Hijazi[1], Mohammed A. Alnafeai[1], Rami A. Hawsawi[1], Waheed M. Bakkawi[1], Mazin M. Kheyami[1], and Ibrahim R. Alzahrani[1], MD

[1]Makkah Health Cluster, Alnoor Specialist Hospital, Laboratory and Blood Bank, Makkah, Saudi Arabia
[2]University of Umm Alqura, Faculty of Medicine, Department of Microbiology and Parasitology, Makkah, Saudi Arabia

Correspondence
Dr. Basem A. Jawa
Department of Laboratory and Blood Bank, Alnoor Specialist Hospital, Ministry of Health, Makkah 21955, Saudi Arabia
e-M: bajawa@moh.gov.sa

Submission: 09 Nov. 2024
Accepted: 18 Dec. 2024

Citation
Jawa BA, Alhazmi KA, Shaikh MM, Albulushi MA, Alkhozaee MM, Almalki MD, Filfilan WM, Falemban FH, Alqurashi EN, Hijazi AA, Alnafeai MA, Hawsawi RA, Bakkawi WM, Kheyami MM, and Alzahrani IR. Site-directed mutagenesis in viral glycoprotein and its role in viral diagnostics and therapy. JKAU Med Sci 2024; 31(2): 63-66. DOI: 10.4197/Med.31-2.6.

Abstract

Site-directed mutagenesis introduces precise mutations into viral glycoprotein genes, allowing the study of their structural and functional roles and interactions with host cells. By modifying specific amino acids, researchers can assess the impact on viral entry, replication, and immune evasion. Engineered glycoproteins improve diagnostic assay sensitivity and specificity and facilitate the development of better vaccine antigens. Therapeutically, site-directed mutagenesis aids in designing antiviral drugs targeting specific glycoprotein regions and developing attenuated viral strains for vaccines. Additionally, this approach enhances understanding of viral evolution and adaptation, offering insights into future pandemic threats and aiding preparedness efforts.

Keywords

Mutagenesis, Viral glycoprotein, Site-directed mutagenesis.

INTRODUCTION

Research and development in viral glycobiology have provided a huge impetus for the application of virology. This was due to the discovery of the role of surface glycoproteins of viruses in the processes of their adsorption, penetration into the cell, and their participation as antigens and effectors in the development of the immune response of microorganisms (Shimada, 1996). The surface glycoprotein of a viral particle is a complex molecule formed by a polypeptide with a high or low molecular weight, with glyosidic chains attached to it. Such chains can bind viruses that are capable of causing severe infectious pathologies in humans and animals. These viruses include the herpes simplex virus, feline immunodeficiency virus, monkeypox virus, influenza virus, and Marburg virus (Baranovich et al., 2013).

To elucidate the role of the surface glycoproteins of these viruses in the pathogenesis of viral diseases and their use in diagnostic and therapeutic purposes, site-specific mutagenesis was used. In this study, the issues of improving this method and assessing their results are considered based on personal experimental experience. The site-directed mutagenesis of viral glycoproteins has emerged as a powerful tool for advancing viral diagnostics and therapeutic strategies (Liu, 2008).

This technique allows researchers to introduce specific mutations into genes encoding viral glycoproteins, enabling the study of their structure-function relationships and their interactions with host cells (Braman, 2002). By altering key amino acid residues, scientists can investigate how these changes affect viral entry, replication, and immune evasion. In diagnostics, engineered glycoproteins can be used to develop more sensitive and specific assays for viral detection as well as to create improved antigens for vaccine development (Braman, 2002). Therapeutically, site-directed mutagenesis has facilitated the design of novel antiviral drugs that target specific glycoprotein regions and the development of attenuated viral strains for potential use in vaccines. Furthermore, this approach has contributed to our understanding of viral evolution and adaptation, providing insights into potential future pandemic threats, and guiding preparedness efforts (Schiffer et al., 2012).

VIRAL GLYCOPROTEINS

Viral glycoproteins are integral components of the viral envelope and play a crucial role in mediating viral entry into host cells. These proteins are anchored to the viral membrane and project outward, enabling the virus to interact with specific receptors on the host cell surface (Sanjuán, 2010). Viral glycoproteins are responsible for various stages of the viral life cycle including attachment, penetration, and fusion with the host cell membrane. Understanding the structure and function of viral glycoproteins is essential to develop effective diagnostic tests and therapeutic interventions (Sanjuán, 2010).

Site-directed mutagenesis is a powerful technique for studying protein function by introducing specific amino acid substitutions (Nøhr and Kristiansen, 2003). This method involves altering the code of a gene to create mutant proteins with targeted changes in their sequences. By systematically modifying specific residues within a viral glycoprotein, researchers can investigate the impact of these changes on the protein structure, stability, and function. This approach allows for a detailed understanding of the critical amino acids involved in protein-protein interactions, enzymatic activity, and other essential biological processes (Nøhr and Kristiansen, 2003).

APPLICATIONS OF SITE-DIRECTED MUTAGENESIS

Viral glycoproteins are typically composed of three distinct domains: ectodomain, a transmembrane domain, and cytoplasmic tail (Jeltsch and Lanio, 2002). The ectodomain, which is exposed to the external environment, contains binding sites for host cell receptors and is involved in mediating viral attachment and entry. The transmembrane domain anchors the glycoprotein to the viral envelope, whereas the cytoplasmic tail plays a role in intracellular signaling and viral assembly (Jeltsch and Lanio, 2002).

Viral glycoproteins play crucial roles in various stages of the viral life cycle. Attachment to the host cell is mediated by the binding of the viral glycoprotein ectodomain to specific receptors on the host cell surface (Jeltsch and Lanio, 2002). Following attachment, the

virus undergoes membrane fusion, which allows the viral genome to enter the host cell. The cytoplasmic tail of the viral glycoprotein interacts with host cell proteins, facilitating viral entry and subsequent intracellular events. Additionally, viral glycoproteins can modulate the host immune response, enabling viruses to evade detection and elimination by the immune system. Understanding the molecular interactions between viral glycoproteins and host cell receptors is essential for developing antiviral strategies targeting these critical steps in the viral life cycle (Zhang et al., 2021).

Site-directed mutagenesis is a precise genetic engineering technique that allows the targeted modification of specific nucleotides within a gene. This method involves the use of synthetic oligonucleotides known as primers that contain the desired mutation (Ran et al., 2020; Edelheit et al., 2009). These primers were designed to anneal to the gene of interest and were then extended by DNA polymerase to create a mutant DNA molecule. By introducing specific nucleotide changes, researchers can alter the amino acid sequence of a protein and study its resulting functional consequences. Compared to random mutagenesis, which generates a large number of mutations without control over their location, site-directed mutagenesis offers greater precision and allows for systematic investigation of the effects of specific amino acid substitutions on protein function. This technique has been widely applied in viral glycoprotein research to identify critical residues involved in protein-protein interactions, enzymatic activity, and other essential biological processes. Additionally, site-directed mutagenesis can be used to create mutant viral glycoproteins that are resistant to antiviral drugs, thereby providing valuable insights into the development of novel therapeutic strategies (Ran et al., 2020; Edelheit et al., 2009).

The fields of site-directed mutagenesis and viral glycoprotein research are rapidly evolving, with several emerging trends. One notable trend is the increasing use of high-throughput mutagenesis techniques, which allow the generation and screening of a large number of mutant proteins in a shorter timeframe. Additionally, advancements in protein structure determination and computational modelling have provided new insights into the structural and functional properties of viral glycoproteins, facilitating the identification of potential drug targets (Ran et al., 2020).

Despite its numerous advantages, site-directed mutagenesis is challenging. One potential issue is the occurrence of off-target mutations, which can introduce unintended changes in the protein sequence and confound the interpretation of the experimental results. Ethical considerations also arise when working with viruses, particularly those with a pandemic potential. It is essential to ensure that research involving these pathogens is conducted in a responsible and ethical manner with appropriate biosafety measures in place (Oka et al., 2011).

Future research directions in this field include the development of more efficient and precise mutagenesis techniques, exploration of novel drug targets within viral glycoproteins, and studying the role of viral glycoproteins in immune evasion and pathogenesis. Additionally, greater emphasis should be placed on understanding the interactions between viral glycoproteins and host cell factors as these interactions are crucial for viral entry, replication, and dissemination. By addressing these challenges and pursuing research directions, scientists can continue to make significant advancements in our understanding of viral glycoproteins and develop innovative therapeutic strategies for combating viral infections (Liun and Naismith, 2008).

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare. All co-authors have seen and agreed with the contents of the manuscript. There are no financial interests to disclose. We certify that the submission is an original work and is not under review at any other publication.

DISCLOSURE

The authors did not receive any form of commercial support, either in the form of compensation or financial assistance, for this case report. The authors have no financial interest in any of the products, devices, or drugs mentioned in this article.

REFERENCES CITED

Baranovich T, Wong SS, Armstrong J, Marjuki H, Webby RJ, Webster RG, and Govorkova EA. 2013. T-705 (favipiravir) induces lethal mutagenesis in influenza A H1N1 viruses in vitro. Journal of virology, 87(7), 3741-3751.

Braman J. (Ed.). 2002. In vitro mutagenesis protocols (Vol. 182). Totowa, NJ: Humana Press.

Edelhei O, Hanukoglu A, and Hanukoglu I. 2009. Simple and efficient site-directed mutagenesis using two single-primer reactions in parallel to generate mutants for protein structure-function studies. BMC Biotechnology, 9, 1-8.

Jeltsch A, and Lanio T. 2002. Site-directed mutagenesis by polymerase chain reaction. In Vitro Mutagenesis Protocols, 85-94.

Liu L, and Lomonossoff GP. 2008. Site-Directed Mutagenesis of Whole Viral Genomes. Plant Virology Protocols: From Viral Sequence to Protein Function, 395-404.

Liu H, and Naismith JH. 2008. An efficient one-step site-directed deletion, insertion, single, and multiple-site plasmid mutagenesis protocol. BMC Biotechnology, 8, 1-10.

Nøhr J, and Kristiansen K. 2003. Site-directed mutagenesis. Protein Misfolding and Disease: Principles and Protocols, 127-131.

Oka T, Murakami K, Wakita T, and Katayama K. 2011. Comparative site-directed mutagenesis in the catalytic amino acid triad in calicivirus proteases. Microbiology and immunology, 55(2), 108-114.

Ran G, Chen X, Xie Y, Zheng Q, Xie J, Yu C, and Ling C. 2020. Site-directed mutagenesis improves the transduction efficiency of capsid library-derived recombinant AAV vectors. Molecular Therapy-Methods & Clinical Development, 17, 545-555.

Sanjuán R. 2010. Mutational fitness effects in RNA and single-stranded DNA viruses: common patterns revealed by site-directed mutagenesis studies. Philosophical Transactions of the Royal Society B: Biological Sciences, 365(1548), 1975-1982.

Shimada A. 1996. PCR-based site-directed mutagenesis. In Vitro Mutagenesis Protocols, 157-165.

Schiffer JT, Aubert M, Weber ND, Mintzer E, Stone D, and Jerome KR. 2012. Targeted DNA mutagenesis for the cure of chronic viral infections. Journal of Virology, 86(17), 8920-8936.

Zhang K, Yin X, Shi K, Zhang S, Wang J, Zhao S, and Deng W. 2021. A high-efficiency method for site-directed mutagenesis of large plasmids based on large DNA fragment amplification and recombinational ligation. Scientific Reports, 11(1), 10454.