Vol. 13 No. 2 February 2024 – May 2024

MEDICINUS

JURNAL KEDOKTERAN
UNIVERSITAS PELITA HARAPAN

Diterbitkan oleh Fakultas Kedokteran, Universitas Pelita Harapan, Indonesia
ISSN 1978 - 3094
E ISSN 2622 - 6995

MEDICINUS

Journal of Faculty of Medicine
University of Pelita Harapan

Vol.13 No. 2
February 2024 – May 2024
ISSN 1978-3094
E-ISSN 2622-6995

Clinical Article

Case Report

Review Article

Address

FK-UPH Building 2nd floor
Boulevard Jendral Sudirman
Lippo Karawaci, Tangerang
phone (021) 54210130-54210131
e-mail:
medicinus.fk@uph.edu

Publish

February – June – October

Comparison of 2009 and 2011 WHO Guidelines, and Scoring Models for Adult Hospitalized Dengue Infection: A Single Observation in Tangerang, Indonesia

Veronica Wiwing[1], Josephine Japutri[2], Neneng Suryadinata[2]

[1] Department of Microbiology, Faculty of Medicine, University of Pelita Harapan, Tangerang, Indonesia, Jendral Sudirman Boulevard, Lippo Karawaci, Tangerang, Banten, Indonesia 15811
[2] Faculty of Medicine, University of Pelita Harapan, Tangerang, Indonesia, Jendral Sudirman Boulevard, Lippo Karawaci, Tangerang, Banten, Indonesia 15811

Citation: Wiwing Veronica, Japutri Josephine, Suryadinata Neneng. Comparison of 2009 and 2011 WHO Guidelines, and Scoring Models for Adult Hospitalized Dengue Infection: A Single Observation in Tangerang, Indonesia. Medicinus. 2024 February. 13(2): 93-98.
Keywords: Dengue; WHO dengue diagnostic; Scoring model
Correspondance: Veronica Wiwing
E-mail: veronica.wiwing@uph.edu
Online First: February 2024

Abstract

Background: World Health Organization (WHO) published a dengue guideline in 2009 and in 2011 by WHO-SEARO. However, many of dengue cases in early phase do not meet all the criteria by WHO classification. Because of this condition there is a scoring model that was published in 2015, that might help in primary health care. Therefore, a study to compare those diagnostic tools especially in adult dengue patients in Banten is needed.

Aims: This study is to know the comparison between 2009 version and 2011 version of dengue diagnostic guidelines by WHO and scoring model version.

Methods and Material: This study used a descriptive method with a cross-sectional design at 60 adult dengue patients. Each patient is grouped according to diagnostic tools' classification and will be analyzed using Chi-square.

Results: results are grouped according to the WHO diagnosis from 2009 and 2011, presumptive model and probable models where there are 46 (77%), 48 (78%), 31(52%), and 15 (25%) of patients diagnosed with dengue infection. Overall, the diagnosis made by the 2009 WHO and the probable models has the most superior sensitivity and specificity values of 84,6% and 25%, and 82,4% and 97,7% respectively compared to other diagnostic tools. However, from the results of positive predictive values, probable models have a higher percentage than the 2009 WHO diagnosis.

Conclusions: probable model is more sensitive and specific than other diagnostic results. These conclude that probable model is best tool for dengue infection screening in early phase of infection.

Introduction

Dengue illness is a viral disease caused by dengue virus of Flavivirus genus, Flaviviridae family that has four serotypes (DENS-1, DENV2, DENV-3, DENV-4) and spread by *Aedes aegypti* mosquito.[1],[2],[3] For the past 50 years, dengue cases had increase by 30 times with 50 million cases each year and 2.5 million people lived in endemic area.[4],[5] According to World Health Organization (WHO), Asia Pacific has contributed 75% of dengue cases in the world and Indonesia as the second country with the most cases among 30 other endemic countries since 2004 to 2010.[5] In 2017 based on Ministry of Health of the Republic of Indonesia, there were 68.407 and 493 of dengue cases and DHF-associated deaths in Indonesia with 26,12 per 100,000 person-years and 0,72% of incidence rate (IR) and case fatality rate (CFR), respectively.[6] The WHO has published new dengue classification in 2009 that was revised from 1997.[4,7] Then in 2011 WHO-SEARO published their dengue guidelines with different classification.[5,8] Previous study in

2015 also published new screening tool in a form of scoring model with two types of model such as presumptive and probable model. This scoring model was designs to diagnose dengue infection in the early phase of illness with limited resources of laboratory facilities in Indonesia.[9] Early studies in 18 countries also showed that 2009 WHO dengue classification has a higher level of accuracy compared to 1997 and 2011 WHO dengue classification.[10],[11],[12] However, 2009 WHO dengue classification is still rarely used in diagnosing dengue illness.[13] This study used the Standard F Dengue IgM/IgG FIA™ and Standard F Dengue NS1 Ag FIA™, which are fluorescence immunoassays from SD Biosensor. This examination is used to obtain IgM, IgG, and NS-1 antigen to be used as a diagnosis of dengue. Therefore, this studied was to compared diagnosis guidelines of 2009 and 2011 WHO dengue classification and scoring model at adult patients in Siloam Teaching Hospital, Banten, Tangerang.

Subjects And Methods

Data source

The study has passed the ethical review from local institutional ethical committee with number of 192/K-LKJ/ETIK/XI/2019 and was conducted from November 2019 to June 2020 in the Faculty of Medicine, Universitas Pelita Harapan. This study used a descriptive method with a cross-sectional design on 60 adult dengue patients with purposive sampling in Siloam Teaching Hospital. Clinical data such as the patient's sign and symptoms and biological materials were collected through appropriate informed consent and were anonymized. Adult patients (age 18-59-year-old) with fever (≥ 37.5 °C) and thrombocytopenia (≥150 000 cell/mcL), were eligible to participate. Patients with a history of autoimmune disease or immunologic/hematologic disorder were excluded.

Case definition and criteria

The 2009 WHO classifies dengue infection into two groups: uncomplicated and severe. Severe cases are linked to excessive hemorrhage, organ impairment, or severe plasma leakage, and the remaining cases are considered uncomplicated.[4] Meanwhile, according to 2011 WHO classification, dengue cases divided into Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF). DHF was further subdivided into grades I-IV, where grade III – IV are considered as Dengue Shock Syndrome (DSS).

- Grade I (DHF I): Fever with bleeding manifestation and evidence of plasma leakage.
- Grade 2 (DHF II): Fever with spontaneous bleeding.
- Grade 3 (DHF III): Clinical sign of circulatory failure or shock.
- Grade 4 (DHF IV): Profound shock with undetectable blood pressure and pulse.[5]

The Scoring Model is a scoring formed using the Roc tab analysis method which gives the results of two models, namely presumptive dengue illness and probable dengue illness with a total value of total ≥14 dan ≥7, respectively. The presumptive model variables are duration of fever, torniquet test, myalgia, monocyte, white blood cell, and thrombocytes examination results. The probable model also have the same variables however, this model is distinguished by the presence of laboratory test result of NS-1 antigen[9].

Standard F Dengue

All eligible patients were screened with Standard F Dengue IgM/IgG FIA™ and NS1 Ag FIA™. Positive NS1 Ag FIA or IgM detection results following either positive or negative IgG FIA results were confirmed dengue. Each patient is grouped according to diagnostic tools' classification and will be analyzed using Chi-square to determine the sensitivity, specificity, likelihood ratio, and negative and predictive value of each diagnostic tool. The sensitivity and specificity value of WHO diagnosis from

2009 and 2011, the presumptive model, and probable model were compared to decide the best screening tool for dengue diagnosis. The examination was carried out according to the protocol provided by SD Biosensor as follows:

Standard F Dengue IgM/IgG FIA™:

1. Store the probe and sample at 15-30°C for at least 30 minutes
2. Set up the Standard F analyzer and select "standard test" mode.
3. Prepare 10μl of sample serum / plasma / blood on the standard black line Ezi Tube^{+}.
4. Enter the sample that has been prepared on the inspection tool.
5. Add 3 drops of dilution liquid to the probe
6. Press "start" to start diagnosis
7. The inspection tool will process and provide results after 15 minutes.

Standard F Dengue NS1 Ag FIA™

1. Store the probe and sample at 15-30°C for at least 30 minutes.
2. Set up the Standard F analyzer and select "standard test" mode.
3. Prepare 100μl of sample serum / plasma / blood sample with a dropper and mix it with dilution liquid.
4. Enter the sample that has been prepared on the inspection tool.
5. Add 3 drops of mixed dilution liquid.
6. Press "start" to start diagnosis.
7. The inspection tool will process and provide results after 15 or 5 minutes on samples that have a strong positive result.

Result

There were 60 dengue infection cases were included in this study. The demographic characteristic, patients' clinical features, and laboratory results such as hematocrit, white blood cell, thrombocyte, neutrophil, and monocyte level were shown in **Table 1.** There are 32 (53%) male and 28 (47%) female patients with an average age of 34,57-year-old. Nausea/vomiting, myalgia, arthralgia, and anorexia were the most common associated symptoms with acute fever. The laboratory results show the mean hematocrit level of 40,29%, indicating that there were no patients with plasma leakages condition. In the early phase, the mean total white blood cell (WBC) count was 4741± 2109,739 cell/mcL and thrombocyte count was 120133 ± 59488 cell/mcL. Moreover, the mean band and segment neutrophils count was 2,75±0,728% and 63±14,924% respectively, and the mean monocyte count was 6,82±1,578%.

Table 1. Demographic results, patients' clinical features, and laboratory results of the participants

Characteristics	N[a]	Min	Max
Demographic information			
a. Age (year)	34,57±12,24	18	59
b. Male	7		
c. Female	32(53)		
Clinical features	28(47)		
Fever			
Retro-orbital pain	58(97)		
Nausea/vomiting	24(40)		
Myalgia	46(77)		
Arthralgia	46(77)		
Anorexia	42(70)		
Constipation	42(70)		
Abdominal pain	8(13)		
Sore throat	22(37)		
Redness	25(42)		
Hepatomegaly	15(25)		
Bleeding manifestation	1(2)		
a. Petechiae			
b. Epistaxis	9(15)		
c. Gastrointestinal	2(3)		
bleeding	2(3)		
d. Gum bleeding	5(8)		
e. Hematoma	2(3)		
Laboratory results		23	57
Hematocrit (%)	40,29±6,326	138	12500
Total WBC count	4741±2109,7	9	27600
(cell/mcL)	39	600	0
Thrombocyte count	120133±594	0	5
(cell/mcL)	88	2	87
Band neutrophils count	2,75±0,728	18	10
(%)	63±14,924	2	
Segment neutrophils	6,82±1,578		
count (%)			
Monocyte count (%)			

*Data presents as n (%) or mean ± standard deviation

All dengue patients are grouped based on WHO diagnosis from 2009 and 2011, presumptive and probable model. There are 46 (77%) patients were diagnosed with dengue infection using WHO diagnoses from 2009 where 24 (47%) patients classified as dengue without warning signs and later 22 (37%) patients classified as dengue with warning signs. On the other hand, based on 2011 WHO there are 47 (78%) patients diagnosed with dengue infection, 28 (47%) of them are classified as dengue fever and 19(32%) patients classified as dengue hemorrhagic fever. Diagnosis by presumptive and probable model shows 31(52%) and 29(48%) patients are positive with dengue infection. All diagnosis results were compared to Standard F Dengue IgM/IgG FIA and NS1 Ag FIA test results to obtain sensitivity and specificity value, log likelihood ratio (LLR), and negative and positive predictive value. The 2009 WHO classification had a sensitivity and specificity of 84,6% and 25,5% respectively. These results have a value of 23,9% and 85,7% for positive predictive value (PPV) and negative predictive value (NPV), and LLR of 0,428. On the other hand, 2011 WHO classification had the sensitivity value of 84,6% and specificity value of 23,4%. The PPV, NPV, and LLR value of 2011 WHO are 23,9%, 84,6%, and 0,522. Furthermore, the comparison between the presumptive model and the results of the Standard F Dengue NS-1 and/or IgM and IgG examination showed a sensitivity of 61.5% and a specificity of 51.1% with PPV, NPV, and LLR, namely 25.8%, 82.8%. , and 0.419, respectively. Next, the probable model has a sensitivity and specificity of 82.4% and 97.7%. The sensitivity and specificity had a PPV and NPV of 93.3% and LLR of 0.00. The comparison between the proposed scoring model and WHO classification is presented in **Table 2.**

Table 2. Comparison Dengue Diagnostic Value of WHO Classification and Scoring Model

	NS-1 (+)/(-) and/or IgM (+) and IgG (+)/(-)				
	Sen (%)	Spe (%)	PPV (+)	NPV (-)	LLR
2009 WHO Dengue Classification	84,6	25,5	23,9	85,7	0,428
2011 WHO Dengue Classification	84,6	23,4	23,4	84,6	0,522
Presumptive Model	61,5	51,1	25,8	82,8	0,419
Probable Model	82,4	97,7	93,3	93,3	0,00

Sen = Sensitivity, Spec = Specivicity, PPV = Positive Predictive Value, NPV = Negative Predictive Value, LLR = Log Likelihood Ratio

Discussion

From those comparisons, the 2009 WHO classification and probable model had a sensitivity of 84,6% and 82,4%, and specificity of 25,5% and 97,7% respectively. These values showed that the 2009 WHO and probable model were higher than the other diagnostic results. Previous studies about these scoring models also determined that 2009 WHO and probable had higher results of sensitivity and specificity.[9],[13],[14] However, it should be seen from the ppv based on WHO diagnosis guidelines, although it has high sensitivity and specificity value, however, the PPV is low at 23.9% compared to the probable model which has a PPV of 93.3% On the other hand, the llr of the probable model is 0,00, in which the diagnostic tools such as Standard F Dengue IgM/IgG FIA and NS1 Ag FIA test are still needed for patients' early diagnosis in dengue infection. In addition, keep in mind that the probable scoring model itself also has a variable of NS-1 antigen examination results. This diagnostic study shows that the probable model could predict dengue illness better than 2009 and 2011 WHO classification, and presumptive model. However, diagnostic tools such as the serologic test of Standard F Dengue IgM/IgG FIA and NS1 Ag FIA test are still needed to further diagnose dengue illness.

Conclusion

Probable model scoring type tool has the highest sensitivity and specificity values than other diagnostic results. In conclusion, the probable model is the best tool for dengue infection screening in early phase of infection. However, serologic test of Standard F Dengue IgM/IgG FIA and NS1 Ag FIA are still needed in determining the diagnosis of dengue illness.

Conflict of Interests

The authors declare no conflict of interests and approve for this manuscript.

References

1. World Health Organization. Dengue. 2016. Available from https://www.who.int/immunization/diseases/dengue/en/

2. Gubler DJ. Dengue and Dengue Hemorrhagic Fever. Clin Microbiol Rev. 1998; 11(3):480-496. https://doi.org/10.1128%2Fcmr.11.3.480

3. World Health Organization. Promoting dengue vector surveillance and control. 2018. Available from: https://www.who.int/activities/promoting-dengue-vector-surveillance-and-control

4. World Health Organization. Dengue Guidelines for Diagnosis, Treatment, Prevention, and Control. 2009. https://www.who.int/publications/i/item/9789241547871

5. World Health Organization Regional Office of South East Asia. Comprehensive Guidelines for Prevention and Control of Dengue and Dengue Haemorrhagic fever. 2011. https://www.aedescost.eu/sites/default/files/2023-01/WHO_SEA-Comprehensive_Guidelines_Prev%26Control_Dengue-2011.pdf

6. Kementrian Kesehatan RI. InfoDatin Situasi Demam Berdarah Dengue. 2018.

7. Hadinegoro S. The revised WHO dengue case classification: does the system need to be modified? Paediatrics and Inernational Child Heath. 2012; 32(SUPPl):33-38. https://doi.org/10.1179%2F2046904712Z.00000000052

8. Nujum Z, Thomas A, Vijayakumar K, Nair R, Pillai R, et al. Comparative performance of the probable case definitions of dengue by WHO (2009) and the WHO-SEAR expert group (2011). Pathogens and Global Health. 2014; 108(2):103-110. https://doi.org/10.1179%2F2047773214Y.0000000131

9. Cucunawangsih, Dewi BE, Sungono V, Lugito NPH, Sutrisna B, et al. Scoring Model to Predict Dengue Infection in the Early Phase of Illness in Primary Health Care Centre. iMedPub Journals. 2015; 6(4):1-8.

10. Barniol J, Gaczkowski R, Barbato, Cunha R, Salgado D, et al. Usefulness and applicability of the revised dengue case classification by disease: multi- centre study in 18 countries. BMC Infectious Disease. 2011; 11(106):1-12. https://doi.org/10.1186/1471-2334-11-106

11. Pothapregeda S, Sivapurapu V, Kamalakannan B, Thulasingham M. Validity and Usefulness of Revised WHO Guidelines in Children with Dengue Fever. Journal of Clinical and Diagnostic Research. 2018; 12(5):SC01-SC05. https://doi.org/10.7860/JCDR/2018/32021.11528

12. Kalayanarooj S. Dengue classification: current WHO vs the newly suggested classification for better clinical application?. Journal of Medical Association of Thailand = Chotmaihet thangphaet. 2011; 94 Suppl 3. Available form; https://www.mendeley.com/catalogue/dengue-classification-current-vs-newly-suggested-classification-better-clinical-application-1/.

13. Van De Wed C, Van Gorp E, Supriatna M, Soemantri A, Osterhaus A, et al. Evaluation of the 2009 WHO Dengue Case Classification in an Indonesian Pediatric Cohort. American Journal of Tropical Medicine and Hygiene. 2012; 86(1):166-170. https://doi.org/10.4269%2Fajtmh.2012.11-0491

14. Macedo GA, Gonin MLC, Pone SM, Cruz OG, Nobre FF, Brasil P. Sensitivity and specificity of the World Health Organization dengue classification schemes for severe dengue assessment in children in Rio de Janeiro. PloS ONE. 2014; 9(4). https://doi.org/10.1371/journal.pone.0096314

Difference Disease Knowledge Level in Type 2 Diabetes Mellitus Patients at Siloam Lippo Village General Hospital

Shirley Ivonne Moningkey [1], Iegreat Aprilyanri[1], Wahyuni Lukita Atmodjo[2]

[1]Department of Public Health and Family Medicine, Faculty of Medicine, Pelita Harapan University, Karawaci, Tangerang, Banten, Indonesia
[2]Department of Anatomy, Faculty of Medicine, Pelita Harapan University, Karawaci, Tangerang, Banten, Indonesia

Citation: Moningkey Shirley, Aprilyanri Iegreat, Atmodjo Wahyuni. Difference Disease Knowledge Level in Type 2 Diabetes Mellitus Patients at Siloam Lippo Village General Hospital. Medicinus. 2024 February. 13(2):99-105.
Keywords: Type 2 Diabetes Mellitus; Knowledge of DM; Glycaemic levels; MDKT
Correspondance: Shirley Ivonne Moningkey
E-mail: smoningkey@yahoo.com
Online First: February 2024

Abstract

Background: In Indonesia, Diabetes Mellitus (DM) with complications is the third leading cause of death. Risk of complication increases tremendously in uncontrolled diabetes. The level of knowledge is one of the factors affect glycaemic control. However, little study has been done regarding the difference in disease knowledge level in type 2 DM patients. This study aims to find out the difference between the level of DM knowledge and glycaemic control in type 2 diabetes mellitus patients at Siloam Lippo Village General Hospital.

Methods: Analytical observational with a cross-sectional study was conducted. 46 type 2 diabetes mellitus patients qualified for the inclusion criteria and were given the self-administered Michigan Diabetes Knowledge Test (MDKT) for General Knowledge Part (GKP) questionnaire and HbA1c test results taken in the past six months to evaluate glycaemic control. Purposive sampling method was used in this study for data collection. Student T-test was done to measure the difference with 95% significancy.

Result: In 46 samples were shown that 60.90% women, a majority in the 50-59 age group, and 65.20% with more than 5 years of DM history. Among 46 samples, 26 have uncontrolled glycaemic with a mean score of 6.65 ± 1.83 in knowledge, and 20 have controlled glycaemic with a mean score 7.80 ± 1.61. Student T-test showed significant difference in level of knowledge between controlled and uncontrolled glycaemic levels with p = 0.032.

Conclusions: It is concluded that there is a difference in disease knowledge level in type 2 DM patients at Siloam Lippo Village General Hospital.

Introduction

Diabetes Mellitus (DM) is estimated to affect 537 million adults globally, thus making it a main concern in public health today.[1] These numbers have persistently increased since 1980, with a total of 108 million patients diagnosed with DM. Type 2 DM amounted to a staggering 98% making up a ratio of 9:1 compared to type 1 DM.[1,2] In 2014, it was found that 8.5% of adults aged above 18 had diabetes. Not only that, DM has affected a large portion of society today, but based on the data in 2019, diabetes was the direct cause of 1.5 million deaths, and 48% of all deaths due to diabetes occurred before the age of 70 years.[3] Currently, Indonesia is placed fifth with the greatest number of people with diabetes at 20 – 79 years old in the year 2021, amounting to 19.5 million. These numbers are expected to inflate by 2045 to an astonishing 16.7 million. Based on IDF 2021, it is found there 73.7% of type 2 DM patients remain underdiagnosed.[1-5] Variation of DM's prevalence rate depends on several factors, including genetic susceptibility, social risk factors such as level of activity, and intrauterine growth.[5,6]

DM is a complex, chronic disease that is caused by metabolic disorders. Several factors in type 2 DM patients, contribute to insulin resistance; thus, uncontrolled blood glucose levels result in

the early onset of DM complications.[5] DM contributes to one of the main causes of blindness, renal failure, heart attack, stroke, and lower limb amputation.[6-8] Several factors affect the level of glycaemic in type 2 DM patients, including medication adherence, age, diagnosis duration, knowledge level, and medication regimen.[9] Therefore, the primary goal in managing diabetes mellitus is to maintain a near-normal glycaemic level.[10] HbA1c (Glycosylated haemoglobin), the primary target in determining a controlled DM, is formed by the nonenzymatic covalent addition of glucose moieties to haemoglobin in red cells. HbA1c is used as an index to indicate the average blood glucose level during the past three months and is little affected by day-to-day variations.[6] Consequently, the factors affecting glycaemic control are the major therapeutic target for preventing of organ damage caused by DM.

Poor disease knowledge is pivotal in the management of DM; thus, the self-care that plays a big role in disease management is often poorly done. The increased knowledge disparity regarding diabetes affects glycaemic control and disease control, whereby poor glycaemic control leads to increased mortality and early-arise of DM-related complications, as discussed above. [11-15] Knowledge of the disease plays a vital role in the management of type 2 DM patients. Although knowledge is an important part of disease care, educating patients regarding the knowledge of the disease itself is often neglected.[12] Many questionnaires have been formed to test disease knowledge of DM. In this study, MDKT is going to be used. The MDKT is a valid and reliable measuring tool for assessing DM knowledge. To assess knowledge of DM, the General Knowledge Part (GKP) is used, which consists of 14 questions regarding DM knowledge, namely 6 questions about food and nutrition, 2 questions about blood tests, 1 question about physical activity, 2 questions about self-care and, 3 questions about complications.[16]

Knowledge of disease plays a vital role in the management of type 2 DM patients and is an important part of the disease care, but educating patients regarding the knowledge of the disease itself is often neglected. [10,17,18] Although the previous study by Thanh and Tien,[19] reported that there was a difference in MDKT results between educated and not educated DM patients, however, the study between the difference in the level of knowledge and glycaemic control in controlled and uncontrolled type 2 DM patients has not yet been done clearly.

Material And Methods

This research had gone through ethical clearance that was released by Universitas Pelita Harapan Ethics Committee on 8th January 2020; 079/K-LKJ/ETIK/I/2020. A cross-sectional study was conducted to assess the level of knowledge and level of glycaemic control among type 2 DM patients at Siloam Lippo Village General Hospital from January 2020 to March 2020.

Patients were selected through purposive sampling and asked for sex, age, educational status, occupational status, and duration of diabetes mellitus. The level of knowledge was then assessed using the General Knowledge Part of Michigan Diabetes Knowledge Test 2 (MDKT 2), and the level of glycaemic control is based on the HbA1c level (NGSP) for the last 6 months that is measured in the hospital's laboratory. Patient with <7% of HbA1c is deemed as controlled glycaemic level. The MDKT 2 questionnaire will consist of self-care, diabetes, symptoms of diabetes complications, and blood glucose examinations.[12-15.]

The inclusion criteria in this study are type 2 diabetes patients admitted to outpatient clinics with HbA1c results in the past 6 months. Meanwhile, the exclusion criteria include patients with a mental disorder or change in consciousness that could hinder the accuracy of the knowledge assessment results.

Data collected will then be analysed for normality distribution using Shapiro-Wilk and Student T-test to obtain the mean difference with 95% significancy.

Result

From 59 samples obtained, 13 did not qualify for the criteria, thus only 46 samples were obtained in total that were included in this study. Sample characteristics of this study that qualified for the criteria are stated in table 1.

Table 1. Samples' Characteristics

Characteristic		N=67	Percent- age (%)
Gender	Male	18	39.10
	Female	28	60.90
Body Mass Index (BMI)	Normal	23	50.00
	Type 1 Obesity	19	41.30
	Type 2 Obesity	4	8.70
Education Status	Elementary	40	59.70
	Middle school	6	13.00
	High school	17	37.00
	Tertiary	19	41.30
Age Groups (years)	< 40	4	8.70
	40 – 49	3	6.50
	50 – 59	21	45.70
	60 – 69	13	28.30
	70 – 79	5	10.90
History of Diabetes (years)	< 5	16	34.80
	5 – 10	17	37.00
	> 10	13	28.20

Table 1 shows patients characteristics, among 46 patients, dominated by female amounting at 28 samples (60.90%). Most of the samples with normal body mass index as many as 23 samples (50%) followed by type 1 obesity as many as 19 samples (41.30%). The education level of the samples showed around 19 samples (41.30%) with a tertiary education background, followed by high school 17 samples (37.00%). Majority of the samples belongs to 50-59 years old with 21 samples (45.70%), followed by 60 – 69 years old amounting at 13 samples (28.30%). Majority of the samples have a history of diabetes ranging from 5 to 10 years with 17 samples (36.95%), followed by more than 10 years of history at 13 samples (28.26%).

Table 2. Samples' variable characteristics

	Mean	SD	Min	Max
HbA1c	7.55	1.60	4.90	11.20
GKP	7.15	1.81	3	11

Patients' variable characteristics that have been assessed can be found at table 2. Both variables are assessed with numeric data in which mean, standard deviation, minimal dan maximal values are stated. Based on table 2, mean of HbA1c 7.55 ± 1.60 and GKP 7.15 ± 1.81.

Table 3. Samples' knowledge passing rate based on MDKT questionnaire (GKP)

	MDKT Items (GKP)	Percentage (%)
1.	The diabetes diet is: a healthy for most people	69.39
2.	Which of the following is highest in carbohydrate: baked potato	30.61
3.	Which of the following is highest in fat: low fat (2%) milk	44.90
4.	Which of the following is a "free food": any food that has less than 20 calories per serving	16.33
5.	HbA1c is a measure of your average blood glucose level for the past: 6-12 weeks	38.78
6.	Which is the best method for home glucose testing: blood testing	67.35
7.	What effect does unsweetened fruit juice have on blood glucose: raises it	16.33
8.	Which should not be used to treat a low blood glucose: 1 cup diet soft drink	34.69
9.	For a person in good control, what effect does exercise have on blood glucose: lowers it	71.43
10.	What effect will an infection most likely have on blood glucose: raises it	40.82
11.	The best way to take care of your feet is to: look at and wash them each day	46.94
12.	Eating foods lower in fat decreases your risk for: heart disease	69.39
13.	Numbness and tingling may be symptoms of: nerve disease	61.22
14.	Which of the following is usually not associated with diabetes: lung problems	63.27

From 46 samples, most of them are well informed about the questions complications symptoms of DM, ideal diet for DM, checking blood sugar at home and the effect of exercise on blood sugar. Most questions have a passing rate below 50% where there are only 6 questions with a passing rate above >50%.

Table 4. HbA1c categorical data

HbA1c level	N = 46	Percentage (%)
Uncontrolled (≥ 7)	26	56.50
Controlled (< 7)	20	43.50

Glycaemic levels are divided into two categorical groups that is identified as uncontrolled and controlled glycaemic levels. These two groups are based on the latest updated 2021 Type 2 DM guidelines released by Indonesian Endocrinology Association (PERKENI), whereby patients with glycemic levels higher than 7 is identified as uncontrolled and lower than 7 is identified as controlled.[20] As seen on table 4, majority of the samples obtained have uncontrolled glycemic level as many as 26 samples (56.50%), while the samples with controlled glycemic levels were only 20 samples (43.50%). The HbA1c Normality Test using the Shapiro-Wilk method shows a normal distribution, (p-value: 0.008).

Table 5. Differences in knowledge between two groups of glycemic control

	N	Mean	SD	P value
Controlled glycaemic	20	7.80	1.61	0.032
Uncontrolled glycaemic	26	6.65	1.83	

As seen on table 5, mean and p value were obtained after analysed with Student T-test with 95% significancy. In patients with controlled glycaemic mean knowledge of 7.80 ± 1.61 in comparison to uncontrolled glycaemic patients with 6.65 ± 1.83. P value of the difference were found to be 0.032.

Discussion

From the results obtained above, it can be concluded that most of the sample had a history of DM of more than 5 years, as much as 65.20%. Meanwhile, it is found that the amount of uncontrolled diabetes patients is also 56.50%. This result is most likely due to a low level of knowledge regarding a healthy diet. It can also be seen that although most of the sample has a long history of DM, they still lack disease knowledge and uncontrolled diabetes. The result of this study is similar to the study held by Phillips et al., 2018, which found a majority of the samples collected had uncontrolled glycemic level and low levels of knowledge. [21]

This study found a mean HbA1c of 7.55% and a GKP score of 7.15, similar to the study held by Phillips et al., 2018, mean HbA1c level of 9.30% with a mean GKP score of 8.30.[21] Both studies indicated that level of knowledge plays a pivotal role in the management of type 2 DM patients as explained previously. A previous study has also reported multiple variables found to have an association with glycemic control, whereby these variables include employment status, social support, long duration of DM history, and poor knowledge of DM. [22] It is also found that HbA1c level was positively related to medication persistence, this relationship goes both ways since it was also found patients with a high level of medication adherence were found less likely to have poor glycemic control. [23,24]

Based on the patient's knowledge passing rate based on the MDKT questionnaire (GKP), it can be seen only 6 questions were answered with a >50% passing rate, depicting a majority of the questions to be most likely answered incorrectly. Two questions were answered least correctly, questions 4 and 7, regarding diet and nutrition. The results compiled are similar to the study held in Saudi Arabia, whereby questions regarding diet and nutrition were least understood, and questions regarding the effect of exercise and home blood glucose test were most correctly answered. [17]

This study shows a new finding that there was difference level of knowledge between controlled and uncontrolled type 2 DM patients.

Conclusion

There was a significant a different between knowledge about DM in the glycaemic controlled and uncontrolled groups.

References

1. Nam Han Cho (chair) et al. Tenth edition 2021. IDF Diabetes Atlas, 10th edition. 2021;1–150. https://diabetesatlas.org/atlas/tenth-edition/

2. Fan W. Epidemiology in diabetes mellitus and cardiovascular disease. Cardiovascular Endocrinology. 2017;6(1):8-16. https://doi.org/10.1097%2FXCE.0000000000000116

3. Asmelash D, Abdu N, Tefera S, Baynes HW, Derbew C. Knowledge, attitude, and practice towards glycemic control and its associated factors among diabetes mellitus patients. Journal of Diabetes Research. 2019;2019(2593684):1-9. https://doi.org/10.1155/2019/2593684

4. Mirzaei M, Rahmaninan M, Mirzaei M. et al. Epidemiology of diabetes mellitus, pre-diabetes, undiagnosed and uncontrolled diabetes in Central Iran: results from Yazd health study. BMC Public Health. 2020;20(166): 1-9. https://doi.org/10.1186/s12889-020-8267-y

5. Care D, Suppl SS. Classification and diagnosis of diabetes: Standards of medical care in Diabetes 2018. Diabetes Care. 2018;41(Supplement_1):S13–27. https://doi.org/10.2337/dc18-s002

6. Al L et. Harrison's Principles of Internal Medicine, 19th. Harrison's Principles of Internal Medicine. 2015. p. 857–61.

7. Levy NK, Orzeck-Byrnes NA, Aidasani SR, Moloney DN, Nguyen LH, Park A, et al. Transition of a text-based insulin titration program from a randomized controlled trial into real-world settings: Implementation study. J Med Internet Res. 2018;20(3). https://doi.org/10.2196/jmir.9515

8. Aikaeli F, Njim T, Gissing S, Moyo F, Alam U, Mfinanga SG, et al. Prevalence of microvascular and macrovascular complications of diabetes in newly diagnosed type 2 diabetes in low-and-middle-income countries: A systematic review and meta-analysis. PLOS Global Public Health. 2022; 2(6):e0000599. https://doi.org/10.1371%2Fjournal.pgph.0000599

9. Khan MA, Hashim MJ, King JK, Govender RD, Mustafa H, Al Kaabi J. Epidemiology of type 2 diabetes – global burden of disease and forecasted trends. Journal of Epidemiology and Global Health. 2020; 10(1):107-11. https://doi.org/10.2991/jegh.k.191028.001

10. Kakade AA, Mohanty IR, Rai S. Assessment of factors associated with poor glycemic control among patients with Type II Diabetes mellitus. Integrative Obesity and Diabetes. 2018;4(3). http://dx.doi.org/10.15761/IOD.1000209

11. Ahmad NS, Islahudin F, Paraidathathu T. Factors associated with good glycemic control among patients with type 2 diabetes mellitus. J Diabetes Investig. 2014;5(5):563–9. https://doi.org/10.1111%2Fjdi.12175

12. Rosas-Muñoz M, Chávez-Sepúlveda C, Alarcón-Hormazabal M, Godoy F, Vásquez-Aguilar P, Cea-Leiva F. Correlation between glycemic control and knowledge in patients with type 2 diabetes mellitus treated at the Family Health Center of the Araucanía region, Chile. Revista de la Facultad de Medicina. 2018;66(4):589–93. https://doi.org/10.15446/revfacmed.v66n4.60464

13. Bukhsh A, Khan TM, Sarfraz Nawaz M, Sajjad Ahmed H, Chan KG, Goh B-H. Association of diabetes knowledge with glycemic control and self-care practices among Pakistani people with type 2 diabetes mellitus. Diabetes, Metab Syndr Obes Targets Ther. 2019; 12:1409–17. https://doi.org/10.2147%2FDMSO.S209711

14. Al Shareef SM, Al Wabel AA, Al Khathlan MA, Al Khazi AA, Al Maarik AK, Al Garni AM, et al. Glycemic Control in Diabetic Patients in Saudi Arabia: The Role of Knowledge and Self-Management - A Cross-Sectional Study. Glob J Health Sci. 2017;9(12):25. https://doi.org/10.5539/gjhs.v9n12p25

15. Poulimeneas D, Grammatikopoulou M, Bougioukli V, Iosifidou P, Vasiloglou M, Gerama M, et al. Diabetes knowledge among Greek Type 2 Diabetes Mellitus patients. Endocrinologia y Nutrition. 2016;63(7):320–6. https://doi.org/10.1016/j.endonu.2016.04.008

16. Fitzgerald JT, Funnell MM, Anderson RM, Nwankwo R, Stansfield RB, Piatt GA. Validation of the Revised Brief Diabetes Knowledge Test (DKT2). Diabetes Educ. 2016;42(2):178–87. https://doi.org/10.1177/0145721715624968

17. Abouammoh NA, Alshamrani MA. Knowledge about Diabetes and Glycemic Control among Diabetic Patients in Saudi Arabia. Journal of Diabetes Research. 2020 Mar 7;2020:1-6. https://doi.org/10.1155/2020/1239735

18. Alotaibi A, Perry L, Gholizadeh L, Al-Ganmi A. Incidence and prevalence rates of diabetes mellitus in Saudi Arabi: An overview. Journal of Epidemiology and Global Health. 2017 Dec;7(4):211-8. https://doi.org/10.1016/j.jegh.2017.10.001

19. Thanh HTK, Tien TM. Effect of Group Patient Education on Glycemic Control Among People Living with Type 2 Diabetes in Vietnam: A Randomized Controlled Single-Center Trial. Diabetes Therapy. 2021 12(5):1503-1521. https://doi.org/10.1007/s13300-021-01052-8

20. Perkeni. Kriteria Diagnostik DM Tipe 2. Konsensus Pengelolaan dan Pencegahan Diabetes Melitus Tipe 2 di Indonesia. 2021.

21. Phillips E, Rahman R, Mattfeldt-Beman M. Relationship Between Diabetes Knowledge, Glycemic Control, and Associated Health Conditions. Diabetes Spectrum. 2018 Apr 4;31(2):196–9. https://doi.org/10.2337/ds17-0058

22. Dedefo MG, Abate SK, Ejeta BM, Korsa AT. Predictors of poor glycemic control and level of glycemic control among diabetic patients in west Ethiopia. Annals of Medicine and Surgery. 2020;55:238–43. https://doi.org/10.1016%2Fj.amsu.2020.04.034

23. Yu P, Xiao X, Wang L, Wang L. Correlation between self-management behaviors and blood glucose control in patients with type 2 diabetes mellitus in community. J Cent South Univ. 2013;38(4):425– 31. https://doi.org/10.3969/j.issn.1672-7347.2013.04.015

24. Sendekie SK, Netere AK, Kasahun AE, Belachew E. Medication adherence and its impact on glycemic control in type 2 diabetes mellitus patients with comorbidity: A multicenter cross-sectional study in Northwest Ethiopia. PLoS ONE 2022;17(9):e0274971. https://doi.org/10.1371/journal.pone.0274971

Hydrodilatation and Intra-articular Steroid Injection are Both Effective in Management of Frozen Shoulder: A case series

John Christian Parsaoran Butarbutar[1], Albert Riantho[2], Kevin Fidiasrianto[3], Dio Asgira Rizky[4]

[1,2,3,4] *Departement of Orthopaedics and Traumatology, Faculty of Medicine, Pelita Harapan University, Karawaci, Tangerang, Banten, Indonesia*

Citation: Butarbutar J, Riantho A, Fidiasrianto K, Rizky D. Hydrodilatation and Intra-articular Steroid Injection are Both Effective in Management of Frozen Shoulder: A case series. Medicinus. 2024 February. 13(2): 116-120.
Keywords: Hydrodilatation; Intra-articular injection; Frozen shoulder; Adhesive capsulitis
Correspondance: John Christian Parsaoran Butarbutar
E-mail : john.butarbutar@lecturer.uph.edu
Online First : February 2024

Abstract

Background: Frozen shoulder is a common problem in general orthopaedic practice, affecting about 2% of the population. Intra-articular corticosteroids (IA) and hydrodilatation have been reported as more effective among other conservative treatments. However, it is unclear which treatment is superior for frozen shoulder, and the hydrodilatation procedure leads to more discomfort in patients since it involves stretching of joint capsule. In this case series, we present 10 cases of frozen shoulder that were treated with hydrodilatation or IA steroid injection. The purpose of this study is to show the effectiveness of hydrodilatation and IA steroid injection in managing patient with frozen shoulder.

Methods: This study was a retrospective case series of patients who received IA steroid injection or hydrodilatation. Five patients underwent IA steroid injection, and another five patients underwent hydrodilatation. The American Shoulder and Elbow Score (ASES) was used to evaluate each patient before and six months after treatment.

Result: Hydrodilatation and IA steroid injection showed significant improvement in ASES score assessed at 6-month follow-up.

Conclusions: Hydrodilatation and IA steroid injection are both effective to treat frozen shoulder in long term follow up.

Introduction

Frozen shoulder, also known as adhesive capsulitis, is commonly used to describe loss of range of motion in the glenohumeral joint, initially explained by Neviaser.[1] This inflammatory condition cause the fibrosis on glenohumeral joint, followed by gradually progressive stiffness and significant restriction of range of motion (typically external rotation). The etiology of frozen shoulder is still unknown. However, some reasonable risk factors have been identified, such as diabetes mellitus, thyroid disorder, shoulder trauma, stroke, etc.[2] Diabetes mellitus is the most commonly condition associated with frozen shoulder, with diabetic patients having 10%-20% lifetime risk of developing frozen shoulder.[3,4] The epidemiologic prevalence of frozen shoulder is estimated to be slightly greater than 2% in the general population, it affects more women than men, and is more common between the ages of 40 and 60 years.[3]

Several treatments have been recognized and used to reduce pain and increase range of motion faster than the natural course of the disease, this includes oral analgesics, oral or intraarticular (IA) corticosteroids, physiotherapy, manipulation,

and hydrodilatation (distension).[5] In fact, physiotherapy often unsuccessful to treat frozen shoulder, manipulation for frozen shoulder requires anesthesia and associated with higher cost, and also the long term use of oral analgesics can cause many side effects. Among these treatments, IA steroid injection and hydrodilation have been reported to be more effective.[6,7] However, becasue hydrodilatation involves stretching or rupturing the joint capsule to improve glenohumeral mobility,[8] it is more discomfort for the patient. There are still debate over which treatment is better for frozen shoulder between hydrodilatation and IA steroids injection.

In this case series we present 10 cases of frozen shoulder who were treated with IA steroid injection or hydrodilatation. In both treatments, we used the American Shoulder and Elbow Score (ASES) to assess clinical outcome. The purpose of this study is to show the effectiveness of hydrodilatation and IA steroid injection in managing patient with frozen shoulder.

Material and Methods

Patients with frozen shoulder between 2021-2022 were included in this study. The diagnosis of frozen shoulder was obtained from the patients who came to the outpatient clinic with progressive diffuse shoulder pain and limited range of motion, especially 50% loss of external rotation compared to the contralateral side. Ultrasonography and x-ray of the shoulder were done to exclude rotator cuff tear, calcific tendinitis, and osteoarthritis of the shoulder.

Five patients were given IA steroid injection and another 5 patients underwent hydrodilatation. The American Shoulder and Elbow Score (ASES) was used to evaluate each patient before and after treatment. Clinical symptoms were evaluated at 6 months after treatment. We set 6 months for the follow-up evaluation period because this period has already exceeded and also doubled the minimum follow up requirement after the procedure, which should therefore be adequate to assess the result of the injection treatments. The clinical outcome was evaluated using the ASES score, which has been demonstrated to be reliable, valid, and responsive to clinical change, thereby supporting its use as a tool with which to assess functional limitations in patients with shoulder dysfunction.[9]

American Shoulder and Elbow Score (ASES)

The ASES is a standarized, patient-reported outcome measure that evaluates the functional status and pain levels of patients with shoulder and elbow disorder. It includes a physician-rated and patient-rated section, and only the pain visual analog scale (VAS) and 10 functional questions are used to calculate the reported score.[9] The total score has a maximum of 100 point and is equally weighted between pain and function.

The ASES is widely used in clinical research and practice to assess the effectiveness of various treatments and interventions. Moreover, the ASES score has been demonstrated to be a valid and reliable outcome measure for assessing the effectiveness of non-operative outcomes.[9]

Treatment Procedure

Intra-articular Steroid Injection

For IA steroid injection, a posterior approach was used, and the procedure were performed with the patient in the right or left recumbent position. Povidone and alcohol sterilization were performed around the injection site, followed by sterile draping. A 12 mgHz linear array probe is used to identify the glenohumeral joint (Mindray,

Shenzhen). Following skin anesthesia with 1% lidocaine, a 22-spinal needle is inserted medial to lateral in plane with the probe until the tip reaches the target between the labrum and head of the humerus. Then, 5 to 10 cc of normal saline is injected to make sure the fluid spreads intra-articularly. If the needle struck against bone, it was retracted and redirected at a slightly different angle. After that, it was followed by injection of a mixture of 4 mL of water soluble triamcinolone (40 mg), 2 mL of Lidocaine 2%, and 14 mL of NaCl 0.9% slowly. Intra-Articular injection is also confirmed by kick back sensation of the fluid during injection.

Hydrodilatation

For hydrodilatation, the procedure is similar to an IA steroid injection. We continue with normal saline after the steroid injection to distend the capsule further, ideally before the capsule ruptures; that is when resistance begins to loosen during injection, or we stop the injection when the patient can no longer tolerate the discomfort.

Table 1. ASES score of patient pre and post Hydrodilatation

Sex	Age	ASES score pre-Hydrodilatation	ASES score 6 months post Hydrodilatation	Improvement of symptoms based on ASES score
Female	50	30	83	64 %
Male	48	32	81	60%
Female	43	40	84	52%
Male	56	34	85	60 %
Male	56	41	84	51 %

Result

Patients in both groups were assessed for an ASES score at presentation and were evaluated 6 months after the treatment. Table 1 is the ASES score of patients who underwent IA steroid injection. Table 2 is the ASES score of patients who underwent hydrodilatation. The results were drawn for both groups, and it was found that both groups showed a significant improvement of clinical symptoms based on the ASES score. In the IA steroid injection group, there was an average improvement of 66% on the ASES score in 6 months of follow-up (Table 1), whereas the hydrodilatation group showed an average improvement of 57.4% on the ASES score (Table 2).

Discussion

Our study showed, either IA steroid injection or hydrodilatation both showed improvement in ASES score at the six month follow-up. In order to improve the clinical interpretation of ASES score, the minimal clinically important difference (MCID) was used. Ian A. Jones, Ba *et* al. have evaluated the available literature of shoulder MCID and it's shown the average reported MCID values for the ASES score were 15.5 points.[10] Both groups showed improvement in ASES score over the MCID values.

Several studies supported our findings that there is no significant difference between IA steroids injection and hydrodilatation in treatment for frozen shoulder.[7,11,12]

Table 2. ASES score of patient pre and post IA steroid injection

Sex	Age	ASES score pre-IA	ASES score 6 months post-IA	Improvement of symptoms based on ASES score
Male	56	28	80	65 %
Male	66	30	82	63%
Female	53	26	80	67.5%
Male	53	24	84	71%
Female	58	29	81	64%

Lin M-T *et.*al showed that IA was as effective as hydrodilatation in shoulder function improvement and pain reduction, yet hydrodilatation shown better external rotation improvement in medium term follow up but to a minor extent in the long term.[12] Wu WT *et* al. also showed in their study that hydrodilatation achieved similar efficacy as compared with IA steroids injection for the improvement of shoulder function.[7] Moreover, a previous systematic review by Länderman A *et* al. revealed that capsular distension (hydrodilatation) with corticosteroid provides the best overall prospect for short-term pain relief and improvement in range of motion across all time frames for frozen shoulder when compared to IA.[13] Additionally, it has also been shown that hydrodilatation provides further medium-term advantages over IA in external rotation and abduction, yet the period of follow up in this study tends to be shorter.

In long-term follow-up, our clinical series and the studies mentioned above show no clear advantages of hydrodilatation over IA steroid injection. Because hydrodilatation is technically more difficult and frequently causes more pain to patients during the procedure, an IA steroid injection for frozen shoulder is preferable.

This study has some limitations. First, our series was only 10 patients without differentiating the phase of frozen shoulder. Second, we only followed up the patient with ASES score at 6 month follow up without physical examination. However, we believed that 6 months follow up is the strength of this series since most of the studies are short term follow up.[7,11-13] Third, our IA steroids injection procedures might be biased because of the use in significant amounts of normal saline volume that might also distend the capsule. It was used to make sure the fluid was injected intra-articularly and the steroids were spread evenly throughout the capsule.

Conclusion

Hydrodilatation and IA steroids injection are both effective to treat frozen shoulder in long term follow up.

Acknowledgment

The authors declare that there is no conflict of interest in writing this article.

References

1. Neviaser AS, Neviaser RJ. Adhesive capsulitis of the shoulder. J Am Acad Orthop Surg. 2011;19(9):536-42. https://doi.org/10.5435/00124635-201109000-00004

2. Mezian K, Coffey R, Chang KV. Frozen Shoulder. StatPearls. Treasure Island (FL): StatPearls Publishing; 2021.

3. Robinson CM, Seah KTM, Chee YH, Hindle P, Murray IR. Frozen shoulder. J Bone Joint Surg Br. 2012;94-B(1):1-9. https://doi.org/10.1302/0301-620x.94b1.27093

4. Tighe CB, Oakley WS Jr. The prevalence of a diabetic condition and adhesive capsulitis of the shoulder. *South Med J*. 2008;101(6):591-595. https://doi.org/10.1097/smj.0b013e3181705d39

5. Favejee MM, Huisstede BM, Koes BW. Frozen shoulder: the effectiveness of conservative and surgical interventions--systematic review. *Br J Sports Med*. 2011;45(1):49-56. https://doi.org/10.1136/bjsm.2010.071431

6. Challoumas D, Biddle M, McLean M, Millar NL. Comparison of Treatments for Frozen Shoulder: A Systematic Review and Meta-analysis. *JAMA Netw Open*. 2020;3(12):e2029581. https://doi.org/10.1001/jamanetworkopen.2020.29581

7. Wu WT, Chang KV, Han DS, Chang CH, Yang FS, Lin CP. Effectiveness of Glenohumeral Joint Dilatation for Treatment of Frozen Shoulder: A Systematic Review and Meta-analysis of Randomized Controlled Trials. *Sci Rep*. 2017;7(1):10507. https://doi.org/10.1038/s41598-017-10895-w

8. Rymaruk S, Peach C. Indications for hydrodilatation for frozen shoulder. EFORT Open Rev. 2017;2(11):462-468. https://doi.org/10.1302%2F2058-5241.2.160061

9. Michener LA, McClure PW, Sennett BJ. American Shoulder and Elbow Surgeons Standardized Shoulder Assessment Form, patient self-report section: reliability, validity, and responsiveness. *J Shoulder Elbow Surg*. 2002;11(6):587-594. https://doi.org/10.1067/mse.2002.127096

10. Jones IA, Togashi R, Heckmann N, Vangsness CT Jr. Minimal clinically important difference (MCID) for patient-reported shoulder outcomes. *J Shoulder Elbow Surg*. 2020;29(7):1484-1492. https://doi.org/10.1016/j.jse.2019.12.033

11. Kitridis D, Tsikopoulos K, Bisbinas I, Papaioannidou P, Givissis P. Efficacy of Pharmacological Therapies for Adhesive Capsulitis of the Shoulder: A Systematic Review and Network Meta-analysis. *Am J Sports Med*. 2019;47(14):3552-3560. https://doi.org/10.1177/0363546518823337

12. Lin MT, Hsiao MY, Tu YK, Wang TG. Comparative Efficacy of Intra-Articular Steroid Injection and Distension in Patients With Frozen Shoulder: A Systematic Review and Network Meta-Analysis. *Arch Phys Med Rehabil*. 2018;99(7):1383-1394.e6. https://doi.org/10.1016/j.apmr.2017.08.471

13. Lädermann A, Piotton S, Abrassart S, Mazzolari A, Ibrahim M, Stirling P. Hydrodilatation with corticosteroids is the most effective conservative management for frozen shoulder. *Knee Surg Sports Traumatol Arthrosc*. 2021;29(8):2553-2563. https://doi.org/10.1007/s00167-020-06390-x

Association Between Breakfast and Dysmenorrhea in Female College Students at Faculty of Medicine, Pelita Harapan University

Agnes[1], Dwi Savitri Rivami[2]

[1,2] *Faculty of Medicine, Pelita Harapan University, Karawaci, Tangerang, Banten, Indonesia*

Citation: Agnes, Rivami DS, Association Between Breakfast and Dysmenorrhea in Female College Students at Faculty of Medicine, Pelita Harapan University. Medicinus. 2023 February. 13(2):106-115.
Keywords: Breakfast; Dysmenorrhea; Female college students
Correspondence: Dwi Rivami
Faculty of Medicine Universitas Pelita Harapan. Jl. Boulevard Jend.Sudirman, Lippo Karawaci, Tangerang, Indonesia. Tel: +62-21-54210130; Fax: +62-21-54210133;
Email: dwi.rivami@uph.edu
Online First: February 2024

Abstract

Background: Breakfast is an activity of eating and drinking that usually takes place after waking up until 9 A.M. and can fulfill 20-25% of daily nutritional needs. Unsupervised skipping breakfast can affect women's ovarian and uterus dysfunction. Dysmenorrhea is one of the diseases that can reduce productivity and quality of life in women. Previous studies have found a high incidence of dysmenorrhea in female college students who didn't have breakfast. Currently, there isn't much data regarding the association between breakfast and dysmenorrhea in female college students in Indonesia.

Aim: The aim of this study is to discover the association between breakfast and dysmenorrhea in female college students of Faculty of Medicine at Pelita Harapan University.

Methodology: This study is an unpaired analytical comparative categoric design study with a cross-sectional method. Data was collected from 60 college students of Faculty of Medicine at Pelita Harapan University using breakfast questionnaire, WaLLID score for dysmenorrhea, IPAQ-SF and PSS-10 were used to control the confounding variables. The data was analyzed using SPSS 23.

Results: Among 60 samples were collected, the majority of female college students didn't have breakfast habit by 55%. There are 76.7% of female students who suffer dysmenorrhea, 91.7% have moderate and severe stress levels, 85% have high physical activity, and 70% of female college students have normal nutritional status. The results of the analysis showed that the p value > 0.05 for the association between breakfast with dysmenorrhea.

Conclusion: There is no significant association between breakfast and dysmenorrhea.

Introduction

Breakfast is an activity of eating and drinking that usually takes place after waking up until 9 in the morning and can fulfill 20-25% of daily nutritional needs.[1,2] Healthy breakfast that is done regularly can form a more organized lifestyle, increase and fulfills daily nutritional needs and its quality, provides a long satiety effect so that activities can be carried out until noon and also reduces the desire to consume high - calorie snacks.[3,4] Skipping breakfast can increase the risk of eating disorders and affect ovarian and uterus function in adolescent girls.[3] A study in Pakistan found that 55.9% of university students sometimes and rarely eat breakfast.[4] The study conducted by Husnah in Banda Aceh found that most students sometimes eat breakfast (61.5%).[5]

Dysmenorrhea is menstrual pain that is characterized by cramps, intermittent, and centered on the lower abdomen.[6] Dysmenorrhea may occur due to an imbalance of prostaglandin hormones so that the uterus muscles contract strongly and usually occurs at the beginning of menstruation.[7] According to the World Health Organization (WHO), the prevalence of dysmenorrhea in the world is around 16.8 - 81%.[8] Dysmenorrhea can reduce productivity and decrease the quality of life in women.[9] In the United States, almost 90% of women suffer from dysmenorrhea with 10-15% experiencing severe dysmenorrhea while in Indonesia, 54.9% of women suffer dysmenorrhea and around 14% of adolescents do not attend school due to dysmenorrhea.[7,8]

In studies conducted in Japan, Palestine and China, it was found that many female college students who skipped breakfast had a high incidence of dysmenorrhea and irregular menstruation.[10] A study conducted by Husnah in Banda Aceh on high school students showed a significant relationship between breakfast and the severity of dysmenorrhea.[5] There is not much data on the association between breakfast habits and dysmenorrhea in female college students, especially in the Faculty of Medicine, which has more activities than other faculties. Based on the data above, this study aims to discover the association between breakfast and dysmenorrhea in female college students of Faculty of Medicine at Pelita Harapan University.

Methods

Participants and Study Design

The study was conducted using an unpaired categorical comparative analytic study design with a cross-sectional research method. Samples were taken using the convenience sampling method. Data were obtained from questionnaires distributed online from April to May 2023 to all medical college students at Pelita Harapan University through social media. Then the data were sorted based on the inclusion criteria and exclusion criteria, which are not having menarche age less than 12 years and not having a family history of dysmenorrhea. The total number of respondents obtained in this study was 60 respondents.

Research Instrument

Data were obtained from a questionnaire regarding breakfast habits and WaLLID score as a measure of dysmenorrhea. IPAQ-SF and PSS-10 were also used to control for confounding variables. The WaLLID score stands for Working ability, Location, Intensity, Days of pain, Dysmenorrhea which is one of the tools to diagnose dysmenorrhea and can predict sick leave. The WaLLIDD score contains several questions about dysmenorrhea such as: 1) the number of pain locations according to anatomy (none, lower abdomen, lumbar region, lower limbs, and inguinal region), 2) Wong-Baker range of pain (no pain at all, only a little pain, a little more pain, more pain, much more pain, extremely pain), 3) the number of days when menstrual pain is felt (0, 1-2, 3-4, ≥ 5), 4) the frequency of pain until the woman cannot do her activities (never, almost never, almost always, always). Each variable has a score between 0 and 3 with the final score ranging from 0 to 12 points. With a total score of 0 means no dysmenorrhea, scores 1-4 have mild dysmenorrhea, scores 5-7 have moderate dysmenorrhea, and scores 8-12 have severe dysmenorrhea.[25]

Statistical Analysis

Data analysis was conducted using Microsoft Excel 2021 (Microsoft, 2021) for organizing the data and analyzed using

Statistical Package for the Social Sciences 23 (IBM, 2016). Chi square test is used to analyze the association between breakfast and dysmenorrhea in female college students of Faculty of Medicine at Pelita Harapan University. In this research, confounding variables will be controlled by excluding the variables of early menarche age and family history of dysmenorrhea, then stratified analysis is used for the variables of physical activity, stress level, and overnutrition status.

Results

Table 1. Respondents Characteristics

Characteristics	Frequency (n)	Percentage (%)
Breakfast Habits		
Always breakfast	27	45
Never breakfast	33	55
Dysmenorrhea		
Dysmenorrhea	46	76.7
No Dysmenorrhea	14	23.3
Stress Level		
Mild stress	5	8.3
Moderate-Severe stress	55	91.7
Physical Activity		
Low physical activity	9	15
High physical activity	51	85
BMI		
normal nutritional status	42	70
overnutrition status	18	30

Respondents Characteristic

From 60 respondents whose data have met the exclusion and inclusion criteria and have been processed with the SPSS application, it was found that the general description of the characteristics of respondents at Pelita Harapan University Faculty of Medicine students was that the majority of female college students were not used to having breakfast (55%), had a high incidence of dysmenorrhea (76.7%), had moderate and severe stress levels (91.7%), had high physical activity (85%), and had normal nutritional status (70%).

Statistical Test Results

Table 2. Statistical Test Results

Breakfast Habits	Dysmenorrhea		P Value	Odds Ratio (OR)	CI (95%)
	No Dysmenorrhea n (%)	Dysmenorrhea n (%)			
Breakfast	5 (19)	22 (81)			
Never breakfast	9 (27)	24 (73)	0.624	0.606	0.176-2.088

According to the analysis results, the p value is 0.624. The p value > 0.05 indicates that there is no significant association between breakfast and dysmenorrhea in female college students of Faculty of Medicine at Pelita Harapan University.

Statistical Test Results Based on Confounding Factors

Table 3. Statistical Test Results Based on Mild Stress Level

Breakfast Habits	Dysmenorrhea		P Value	Odds Ratio (OR)	CI (95%)
	No Dysmenorrhea n (%)	Dysmenorrhea n (%)			
Breakfast	1 (100)	0 (0)			
Never breakfast	1 (25)	3 (75)	0.819	-	0.733-21.838

Table 4. Statistical Test Results Based on Moderate-Severe Stress

Breakfast Habits	Dysmenorrhea		P Value	Odds Ratio (OR)	CI (95%)
	No Dysmenorrhea n (%)	Dysmenorrhea n (%)			
Breakfast	4 (15)	22 (85)			
Never breakfast	8 (28)	21 (72)	0.270	0.477	0.123-1.824

Table 5. Statistical Test Results Based on Low Physical Activity

Breakfast Habits	Dysmenorrhea		P Value	Odds Ratio (OR)	CI (95 %)
	No Dysmenorrhea n (%)	Dysmenorrhea n (%)			
Breakfast	0 (0)	5 (100)			
Never breakfast	1 (25)	3 (75)	0.906	-	0.757-2.348

Table 6. Statistical Test Results Based on High Physical Activity

Breakfast Habits	Dysmenorrhea		P Value	Odds Ratio (OR)	CI (95%)
	No Dysmenorrhea n (%)	Dysmenorrhea n (%)			
Breakfast	5 (23)	17 (77)	0.157	0.772	0.213-2.797
Never breakfast	8 (28)	21 (72)			

Table 7. Statistical Test Results Based on Normal Nutritional Status

Breakfast Habits	Dysmenorrhea		P Value	Odds Ratio (OR)	CI (95%)
	No Dysmenorrhea n (%)	Dysmenorrhea n (%)			
Breakfast	3 (15)	17 (85)	0.838	0.378	0.083-1.730
Never breakfast	7 (32)	15 (68)			

Table 8. Statistical Test Results Based on Overnutrition Status

Breakfast Habits	Dysmenorrhea		P Value	Odds Ratio (OR)	CI (95%)
	No Dysmenorrhea n (%)	Dysmenorrhea n (%)			
Breakfast	2 (29)	5 (71)	0.263	1.800	0.191-16.980
Never breakfast	2 (18)	9 (82)			

Stress Level

From tables 3 and 4, it is found that the p value in the statistical test results based on mild stress level is 0.819 and at moderate-heavy stress level is 0.270 so it can be concluded that there is no significant association between breakfast and dysmenorrhea in female college students based on stress levels.

Physical Activity

From tables 5 and 6, it is found that the p value in the statistical test results based on low physical activity is 0.906 and at high physical activity is 0.157 so it can be concluded that there is no significant association between breakfast and dysmenorrhea in female college students based on physical activity.

BMI

From tables 7 and 8, it is found that the p value in the statistical test results based on normal nutritional status is 0.838 and at overnutrition status is 0.263 so it can be concluded that there is no significant association between breakfast and dysmenorrhea in female college students based on BMI.

Discussion

Breakfast Overview

The results of this study found that there were more college female students who were not accustomed to having breakfast (55%) compared to college female students who were accustomed to having breakfast (45%). This is in accordance with research conducted by Husnah in Banda Aceh who found that the number of high school students who sometimes do breakfast (61.5%) is greater than students who often have breakfast (38.5%).[5] This is also in line with research conducted in Japan by Tomoko Fujiwara who found that the number of college female students who sometimes or always skip breakfast (95.9%) is much greater than college female students who never skip breakfast (4.1%).[10] There are several reasons that could make college female students do not have the habit of having breakfast such as: schedules that are too busy so they do not have time for breakfast, no one to help prepare breakfast, or having to go out of the house to buy food just for breakfast before doing their lecture activities.[11]

Dysmenorrhea Overview

The results of this study found that there were more college female students who suffered from moderate and severe dysmenorrhea (76.7%) compared to college female students who did not suffer from

dysmenorrhea or only had mild dysmenorrhea (23.3%). This is in line with research conducted by Marini Agustin at As-syafi'iyah Islamic University Jakarta in the Bachelor of Nursing Study Program, found that the number of female students suffering from mild dysmenorrhea was 13 people (21%) and female students suffering from moderate and severe dysmenorrhea were 49 people (79%).[12] Research conducted by Ketut Anita Herdianti, et al. at Udayana University with the Medical Study Program found that female students suffering from dysmenorrhea were 83 people (86.5%) and female students who didn't have dysmenorrhea were 13 people (13.5%).[13] Other factors that can increase the incidence of dysmenorrhea such as poor sleep quality which can affect the secretion of adrenaline and estrogen hormones which can increase muscle contractions in the uterus and exposure to cigarette smoke which contains nicotine that acts as a vasoconstrictor resulting in an increase in prostaglandin levels.[1]

Stress Level, Physical Activity, And BMI Overview

In this study it was found that the number of college female students who experienced moderate and severe stress was higher (91.7%) than female students who did not experience stress or only experienced mild stress (8.3%). This is in line with research conducted at Andalas University Medical Study Program found that the number of students who experienced mild stress was 11.2% with students who experienced moderate and severe stress as much as 88.8%.[15] Based on the results of the study, it was found that the number of college female students who had low physical activity was less (15%) than those who had high physical activity (85%). This is in line with research conducted on health faculty students at Sam Ratulangi University by Lestari E. Liando, et al. with the results found that the number of students who have high physical activity is greater (70.8%) than students with low physical activity (29.2%).[16] In this study it was also found that more female students had normal nutritional status (70%) than female students who had excess nutritional status (30%). This is in line with research conducted on students of the Faculty of Health Sciences at Ibn Khaldun University of Bogor by Chyntia Nurul Adha, et al. who found that the number of students with normal nutritional status was 76.7% and the number of students with excess nutritional status was 23.4%.[17]

Stress in students can occur due to external and internal factors such as the increased responsibility that is felt when moving from a high school student to a college student, the increasing number of tasks that need to be completed or completing the final project which is one of the requirements for a college student to graduate, as well as environmental or cultural changes felt by students studying away from their home.[15] Factors that can affect a person's level of physical activity and nutritional status are gender, age, environmental factors, social support, occupation, physical limitations and economic status.[16] The occurrence of a pandemic can also make a person pay more attention to their health, resulting in a new habit pattern to exercise and care more about clean and healthy living behaviors.[16]

Analysis Results

In this study it was found that there is no significant association between breakfast and dysmenorrhea in female college students. This study is not in line with research conducted by Husnah in Banda Aceh in 2018 which found that there was a significant association between breakfast and the severity of dysmenorrhea. The difference in the results of this study can

occur due to several things such as differences in samples where the research conducted by Husnah was 132 high school students, while the sample in this study was 60 students. In addition, the research conducted by Husnah used the VAS (Visual Analogue Questionnaire Scale) questionnaire to measure the incidence of dysmenorrhea while the researchers used the WaLLID Score questionnaire to measure the incidence of dysmenorrhea.[5] This study is also not in line with research conducted by Tomoko Fujiwara conducted on female students at Kanazawa University, Japan. The difference in the results of this study can occur because the research by Fujiwara was conducted in Japan which has a different culture and food nutrition patterns with female college students in Indonesia, the difference in the number of samples of 3,172 people and the observation period for 1 year can also affect the difference in research results with this study.[10]

The results of the analysis based on the stress level factor showed that there was no significant association between stress levels and dysmenorrhea in Pelita Harapan University Faculty of Medicine students. This is different from research conducted by Bajalan on mental health and dysmenorrhea using a systematic review study where it was found that there was a significant relationship between the majority of psychological disorders such as depression, anxiety and stress with dysmenorrhea although the mechanism is still not clearly known so further research needs to be done.[18] There are also studies that are in line with this study such as research conducted by Maryam which was conducted on students of the Faculty of Medicine, Padjadjaran University and Amran which was conducted at the Faculty of Medicine, Hasanuddin University that found there was no significant association between stress levels and dysmenorrhea.[18] In this study, the majority of respondents had moderate stress levels and severe stress, which may cause an imbalance in the distribution of respondents at other stress levels and ultimately result in the possibility for the results of the analysis in the study to be not significantly related.

Based on the results of the analysis of the relationship between physical activity and dysmenorrhea, there is no significant association between physical activity and dysmenorrhea. This is not in line with research conducted by Karmila on high school students of YLPI Pekanbaru and research by Wati conducted on Midwifery Study Program students of Brawijaya University with respondents who do light activities have a 6.5 times greater chance of suffering from dysmenorrhea than respondents who do moderate activities.[18] Research by Motahari-Tabar conducted with the Randomized Clinical Trial method on students of the Faculty of Medicine at Mazandara University, Iran found that the reduction of dysmenorrhea pain due to the effect of exercise can only be seen if the respondent has done the exercise for two consecutive months and other variables such as type, duration, and intensity of physical activity can also affect the results of research analysis.[18] Research by Dehnavi conducted on students of the Faculty of Medicine, Mashhad University, Iran with the clinical trial method also obtained similar results, namely the results of the analysis there was no significant relationship at the beginning of the study and the end of the fourth week, and was only seen at the end of the eighth week.[18] Therefore, it can be interpreted that the results of the analysis in this study found no significant relationship between physical activity and dysmenorrhea due to only observing physical activity for one week where according to the two studies there

was still no visible change and only visible after about two months.

The results of the analysis found in the analysis of the relationship between nutritional status and dysmenorrhea are that there is no significant association between nutritional status and dysmenorrhea. This result is different from the research conducted by Sophia, et. Al on students of SMK Negeri 10 Medan with the kai squared method and also with research by Cholifah and Hadikasari on Midwifery Study Program students at Muhammadiyah University who used the Fisher's Exact test and found that there was a significant relationship between nutritional status and dysmenorrhea.[19] Research that is in line with this study is research conducted by Vlachou on Nursing Study Program students in Greece where it was found that there was no significant difference between smoking, exercise, BMI, and menstrual duration with dysmenorrhea severity.[20] In several studies it was found that women who have excessive visceral fat tissue usually suffer from severe dysmenorrhea.[20] BMI is one way to measure nutritional status in a person but it is unable to describe the proportion of fat contained in a person's body, so this could be one of the factors affecting the results of the analysis in this study resulting in no significant association between nutritional status and dysmenorrhea.[20]

The odds ratio value in the analysis results based on confounding factors in the variables of mild stress and low physical activity cannot be calculated. This is because if calculating the odds ratio based on the formula, there will be a division with zero which means undefined so that the results of the odds ratio calculation cannot be determined. The presence of zero values in the results of this analysis can be caused by an imbalance in distribution where in this study it was found that 91.7% of the majority of female students experienced moderate-severe stress and 85% of female students had high physical activity so that there was a lack of data on female students who experienced mild stress and female students who had low physical activity. The odds ratio value found in the overweight nutritional status variable is 1.8 which can indicate that there is an indication of risk in that variable. In overnutrition status it was also found that the confidence interval or confidence interval had a fairly wide range from 0.191 to 16.980. The confidence interval on the mild stress variable was also found to be quite wide with a lower limit of 0.733 and an upper limit of 21.838. The factor that can make this happen is because the number of samples used is small or only a few.

Limitation

The limitation found in this study is that it was conducted using an online questionnaire method that has a high risk of bias because it was not supervised by the researcher when the respondent filled out the research questionnaire. In addition, respondents can also answer the questionnaire excessively or minimize the actual results because the questions contained in the research are sensitive or personal for respondents. Another limitation that can be found by researchers is the short period of observation in the questionnaire which is only carried out for seven days while in previous similar studies it tends to be carried out over a period of one month or one year so that it can affect the results of this study. In a study discussing the differences in recall periods, it was found that the longer the period of time for respondents to recall an event, the more the limit of respondents' errors to answer the research questionnaire, so the researcher chose to use a seven-day time limit in the hope that respondents could still remember the activities they did during the week well.[21]

Conclusion

In conclusion, the majority of female college students in Faculty of Medicine at Pelita Harapan University didn't have breakfast habits and experienced dysmenorrhea. Based on the results of the analysis there is no significant association between breakfast and dysmenorrhea in female college students of Faculty of Medicine at Pelita Harapan University.

Acknowledgements and affiliations

The authors express their gratitude to all the team from Faculty of Medicine Pelita Harapan University and to all the subjects who participated in this study.

References

1. Cantika HAK, Ardiaria M, Syauqi A. Peran Makronutrien Dalam Sarapan Terhadap Performa Kognitif Remaja. *Jurnal Kedokteran Diponegoro*. 2017;6(2):611-621. https://doi.org/10.14710/dmj.v6i2.18579

2. Gyna K, Swari Y, Kadek Mulyantari N, Putu W, Yasa S, Klinis DP. Hubungan Melewatkan Sarapan Terhadap Kejadian Overweight dan Obesitas Pada Mahasiswa Fakultas Kedokteran Universitas Udayana. Jurnal Medika Udayana; 11(3):116-121. https://doi.org/10.24843/MU.2022.V11.i03.P18

3. Guevara RM, Urchaga JD, Cabaco AS, Moral-García JE. The Quality of Breakfast and Healthy Diet in School-aged Adolescents and Their Association with BMI, Weight Loss Diets and the Practice of Physical Activity. *Nutrients*. 2020;12(8):2294. http://dx.doi.org/10.3390/nu12082294

4. Zaheer M, Kamran H, Hareem M, Babar N, Ijaz M, Abdul-Rauf M. Assessment of Lifestyle and Dietary Habits and its Effect on Psychological Health Among University Students. *Pakistan Biomedical Journal*. 2023;6(1):2-8. https://doi.org/10.54393/pbmj.v6i01.838

5. Husnah H. Association Between Breakfast to Dysmenorrhea Degree on Students SMAN 2 in Banda Aceh, Indonesia. *World Nutrition Journal*. 2018;2(1):26. http://dx.doi.org/10.25220/WNJ.V02.i1.0006

6. Melliniawati T, Kirana MC, Aenia D, Alfiani RR, Nurzakiyah A, Nasution AS. Pengaruhi Usia Menarche dan Status Gizi Terhadap Kejadian Dismenore Pada Remaja Putri Kelas 3 SMP di Pondok Pesantren XDepok. *Jurnal Bidan Pintar*. 2021;2(2):284-295.

7. Itani R, Soubra L, Karout S, Rahme D, Karout L, Khojah HMJ. Primary Dysmenorrhea: Pathophysiology, Diagnosis, and Treatment Updates. *Korean J Fam Med*. 2022;43(2):101-108. http://dx.doi.org/10.4082/kjfm.21.0103

8. Husna PH, Siwi CSR. Upaya Penurunan Nyeri Dismenorea Dengan Terapi Relaksasi Benson Pada Remaja Putri Di Dusun Balerejo Watuagung Baturetno Wonogiri. *Jurnal Keperawatan GSH*. 2023;12(1):21-24.

9. Agina G, Nurhidayati H, Astutik AW, Nasutiyon M, Lestari CR. Hubungan Riwayat Asupan Zat Gizi Mikro dan Tingkat Stres dengan Penurunan Rasa Nyeri Haid. *Indonesian Journal of Nutrition Science and Food*. 2023;2(1):7-15. https://e-journal.ivet.ac.id/index.php/IJNuFo/article/view/2352

10. Fujiwara T, Ono M, Iizuka T, et al. Breakfast skipping in female college students is a potential and preventable predictor of gynecologic disorders at health service centers. *Diagnostics*. 2020;10(7). http://dx.doi.org/10.3390/diagnostics10070476

11. Putra A, Syafira DN, Maulyda S, Cahyati WH. Kebiasaan Sarapan pada Mahasiswa Aktif. *HIGEIA (Journal of Public Health Research and Development)*. 2018;2(4):577-586. http://dx.doi.org/10.15294/higeia.v2i4.26803

12. Agustin M. Hubungan Antara Tingkat Dismenore dengan Tingkat Stres pada Mahasiswi Akper As-syafi'iyah Jakarta. *Jurnal Afiat*. 2018;4(2):603-611. https://doi.org/10.34005/afiat.v4i02.710

13. Herdianti KA, Wardana NG, Karmaya INM. Hubungan antara kebiasaan olahraga dengan dismenore primer pada mahasiswi pre-klinik Program Studi Pendidikan Dokter Fakultas Kedokteran Universitas Udayana tahun ajaran 2017. *Bali Anatomy Journal*. 2019;2(1):25-29. https://doi.org/10.36675/baj.v2i1.23

14. Nurfadillah H, Maywati S, Aisyah IS. Faktor-Faktor yang Berhubungan dengan Kejadian Dismenore Primer pada Mahasiswi Universitas Siliwangi. *Jurnal Kesehatan Komunitas Indonesia* . 2021;17(1):247-256. https://doi.org/10.37058/jkki.v17i1.3604

15. Rahmayani DR, Gusya Liza R, Syah NA. Gambaran Tingkat Stres Berdasarkan Stressor pada Mahasiswa Kedokteran Tahun Pertama Program Studi Profesi Dokter Fakultas Kedokteran Universitas Andalas Angkatan 2017. *Jurnal Kesehatan Andalas*. 2019;8(1):103-110. http://dx.doi.org/10.25077/jka.v8.i1.p103-111.2019

16. Liando LE, Amisi MD, Sanggelorang Y. Gambaran Aktivitas Fisik Mahasiswa Semester IV Fakultas Kesehatan Mastarakat UNSRAT Saat Pembatasan Sosial Masa Pandemi COVID-19. *Jurnal KESMAS*. 2021;10(1):118-128.

17. Adha CN, Prastia TN, Rachmania W. Gambaran Status Gizi Berdasarkan Lingkar Lengan Atas dan Indeks Massa Tubuh pada Mahasiswi FIKES UIKA Bogor Tahun 2019. *Jurnal Mahasiswa Kesehatan Masyarakat*. 2019;2(5):340-350. https://doi.org/10.32832/pro.v2i5.2523

18. Fasya A, Arjita IPD, Pratiwi MRA, Andika IBY. Hubungan Aktivitas Fisik dan Tingkat Stres dengan Kejadian Dismenorea Primer pada Mahasiswi Fakultas Kedokteran. *Jurnal Ilmiah Permas: Jurnal Ilmiah STIKES Kendal*. 2022;12(3):511-526.

19. Hikma YA, Yunus Moch, Hapsari A. Hubungan Siklus Menstruasi, Kualitas Tidur, dan Status Gizi, Terhadap Dismenore Primer pada Remaja Putri. *Sport Science and Health*. 2021;3(8):630-641. https://doi.org/10.17977/um062v3i82021p630-641

20. Widiyanto A, Lieskusumastuti AD, Sab'ngatun. Hubungan Indeks Massa Tubuh dengan Dismenore. *Avicenna : Journal of Health Research*. 2020;3(2):131-141. https://doi.org/10.36419/avicenna.v3i2.425

21. Kjellsson G, Clarke P, Gerdtham UG. Forgetting to remember or remembering to forget: A study of the recall period length in health care survey questions. *J Health Econ*. 2014;35:34-46. https://doi.org/10.1016/j.jhealeco.2014.01.007

Isolated Abducens Nerve Palsy: A Case Report of Cerebral Pseudocyst of Dorello's Canal

Vivien Puspitasari[1], Josephine Japutri[2], Tracy Solansa[2], Anderson Cenweikiawan[2]

[1] *Department of Neurology, Faculty of Medicine, Pelita Harapan University, Karawaci, Tangerang, Banten, Indonesia*
[2] *Faculty of Medicine, Pelita Harapan University, Karawaci, Tangerang, Banten, Indonesia*

Citation: Puspitasari Vivien, Japutri Josephine, Solansa Tracy, Cenweikiawan Anderson. Isolated Abducens Nerve Palsy: A Case Report of Cerebral Pseudocyst of Dorello's Canal. Medicinus. 2024 February. 13(2): 121-125.
Keywords: Abducens nerve palsy; Dorello's canal; Pseudocyst
Correspondance: Vivien Puspitasari
Department of Neurology, Faculty of Medicine, Pelita Harapan University, Karawaci, Tangerang, Banten, Indonesia, 15811. Phone: +622154010130.
E-mail : vivienpuspitasari@gmail.com
Online First : February 2024

Abstract

Background: Abducens nerve is the second longest intracranial path out of all of the cranial nerves. Abducens nerve pasly had a prevalence 11.3/100.000 and mostly seen in adults. Isolated abducens nerve palsy due to neoplastic lesion are rare and mostly related to skull base tumor.

Case Presentation: We present a case of 57 years old woman with complaints of red right eye and worsening headache associates with diplopia on the right lateral gaze for three days prior admission. She was later diagnosed with isolated abducens nerve palsy associated to neoplasm cause in Dorello's canal. Patient was then discharged with symptomatic therapy and educated for head posturing and avoid triggers.

Conclusions: Abducens nerve palsy is the most common isolated ocular nerve palsy. The incidence of mass formation such as neoplastic lesion or cyst are rare.

Introduction

Of all the cranial nerves, the abducens nerve has the second longest intracranial path. This nerve may be impacted at the petrous apex, cavernous sinus, superior orbital fissure, or eye orbit. Anything that compresses or stretches the nerve can damage the abducens nerve. Therefore, it is critical to pinpoint the lesion of abducens nerve palsy for proper diagnosis and therapy. Isolated abducens nerve palsy due to neoplastic lesion[1,2,3] are rare and mostly related to skull base tumor. Kim et al, found that only 14.3% of the 807 confirmed cases of solitary abducens nerve palsy were connected to neoplasm.[4] Here we described a case of isolated abducens nerve palsy associated with neoplasm lesion in Dorello's canal (petroclival venous confluence).

Case Summary

A 57 years old woman presented at our hospital with a red right eye and worsening headache associated with diplopia on the right lateral gaze for three days prior to admission. The patient claimed that she had experienced these complaints for one year. Upon neurological assessment, there was binocular horizontal diplopia associated with total right abducens nerve palsy. Other physical and blood examinations are normal. There were no signs of elevated intracranial pressure. Investigations including perimetry and optical coherence tomography were carried out, and the results were within normal limits. Magnetic resonance imaging (MRI) with contrast revealed a dilated right dorello's canal with a non-enhanced cystic

lesion on the right side of the superoposterior cavernous sinus (0.8 x 0.5 x 0.5 cm), raising the possibility of a pseudocyst. There is no evidence of either aneurysm or thrombosis of the cavernous sinus. When compared to previous MRI (one year prior), it was comparable.

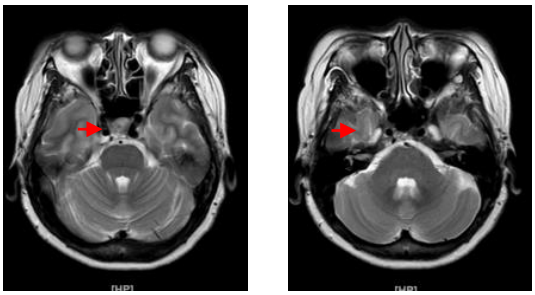

Figure 1. Magnetic Resonance Imaging (MRI) with contrast in T2 sequences revealed a dilated right dorello's canal with a non-enhanced cystic lesion on the right side of the superoposterior cavernous sinus (0,8x0,5x0,5 cm).

Although we were unable to make a definitive diagnosis, we assumed the lesion was benign. Therefore the patient was discharged and continues to be monitored at the outpatient clinic without any operation. Symptomatic therapy was administered, and the patient was educated for head posturing and on how to avoid potential triggers.

Discussion

According to reports, abducens nerve palsy are the most typical isolated ocular motor cranial neuropathy.[3,5] The yearly prevalence of abducens palsy is 11.3/100,000 and mostly seen in adults.[6] The abducens nerve's neuroanatomy is separated into four unique sections: the nucleus, the cisternal part, the cavernous region, and the orbital portion. The abducens nucleus originates from the caudal dorsal pons, below the fourth ventricle. These nerve fibers emerge and leave the nucleus superiorly to form the abducens fascicle in the brainstem, then exits the brainstem at the pontomedullary junction to reach the subarachnoid region. The nerve then turns sharply and rises steeply over the tip of the petrous part of temporal bone towards the clivus within a fibrous sheath called Dorello's canal and enters the inferior dura mater to the posterior clinoid process. Dorello's canal is the gap between the petrous apex and the superolateral section of the clivus, which is superiorly restricted by the petrosphenoidal ligament of Gruber (Gruber's ligament). The components included in this canal are bound together and significantly limit their mobility. The inner meningeal tube that surrounds the nerve severely limits the movement of the abducens nerve within Dorello's canal. Because the abducens nerve is anchored in Dorello's canal, it is vulnerable to straining when intracranial pressure rises for a variety of reasons such as venous congestion, edematous pressure or cancerous infiltrates.[7,8] This nerve then enters the cavernous sinus along with the oculomotor nerve, trochlear nerve, and the first branch of trigeminal nerve. Before following the sphenoidal fissure, it laterally follows the internal carotid artery and medially to the sinus lateral wall. The nerve then enters the orbit via the superior orbital fissure within the tendinous ring, eventually reaching the lateral rectus muscle.[5,7]

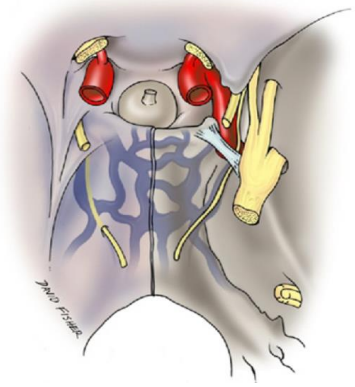

Figure 2. The abducens nerve are illustrated schematically as they proceed through the Dorello canal.

The nerve enters the dura near the beginning of the canal on the left side and goes anteriorly into the cavernous sinus through the opening inferior to the Gruber ligament, as seen on the right side of the picture.[9]

The isolated sixth nerve palsy has different kinds of etiology. The most common underlying pathology is microvascular causes, about 56% cases are caused by vascular disease especially in advanced age. Isolated abducens paralysis is associated with a vascular risk factor such as diabetes (44.4%), hypertension (33.3%), coronary artery disease (13.9%), smoking (13.9%), and hyperlipidemia (8.3%). The other most common etiology are idiopathic (27.2%), inflammatory diseases (8.3%), infections (8.3%) such as Lyme disease, syphilis, tuberculosis and cryptococcus, increased intracranial pressure (8.3%), demyelinating (2.5%), cerebral aneurysm (1.9%) and procedure-related injury (1%). Neoplasm (5.6%) and trauma (4.9%) may also affect the abducens nerve at any point along its course and resulting in sixth nerve palsy.[10] A summary of six retrospective studies on patients with sixth nerve paresis showed 6% to 30% attributed to a miscellaneous group that includes migraine, pseudotumor cerebri, multiple sclerosis. Furthermore 6% to 29% etiology are undetermined, indicating vulnerability of the nerve to conditions which are transient, benign, and unrecognizable.[11] Our case described a female patient with no comorbidities or vascular risk factors, presented with an episode of diplopia and right sided headache. Ischemic, autoimmune, inflammatory, and traumatic causes were ruled out since the history and laboratory tests were inconclusive. However, neuroimaging using contrast MRI was done and showed pseudocyst on the right superoposterior cavernous sinus of the dorello's canal.

MRI is an efficient and safe tool for determining the underlying cause of isolated abducens nerve palsy in patients without identifiable vasculopathic risk factors. It is useful for identifying brain demyelination, infarction, infections, neoplasm, and other mass lesions that are related to nerve palsy.[12,13] In our case, it is suspected that the pseudocyst is likely to cause abducens nerve palsy in our patient by increasing pressure in the dorello's canal and compressing the abducens nerve. This is a rare occurrence of isolated abducens nerve palsy caused by benign neoplastic lesion.[12,13,14]

Patients who develop sixth nerve palsy will often present with binocular horizontal diplopia and notable weakness of ipsilateral lateral rectus muscle leading to a deficit eye abduction of the affected side.[3] In our patients, we saw two common symptoms: diplopia and ipsilateral weakness. The two typical symptoms might lead to side effects that develop in later stages such as headache, vomiting and conjunctival injection that cause eye soreness. Patients also present with a head turn and/or strabismus to maintain binocularity and binocular fusion as coping mechanisms to diplopia in long term findings as seen in our patient.

Therapeutic modalities ranged from conservative treatment, botulinum toxin injections, and surgical treatment in cases of abducens nerve palsy. Due to the size of the pseudocyst that is relatively the same as the previous imaging one year ago, it is concluded that the pseudocyst is not progressive. Treatment that was suggested to the patients are conservative treatment such as patching, and prism therapy could be used to prevent amblyopia in the affected eye. Patching consists of closing each alternating eye for hours using eye patch. Another conservative treatment suggested is prism therapy which requires the placement of a temporary press-on prism on

the lens of the affected eye. Other modalities such as botulism injection could also be given to this kind of cases with residual function of the lateral rectus muscle. Surgical treatment of the lateral rectus muscle called strabismus surgery was also suggested in this case. This surgery consists of resection, recession, or transposition of the eye muscles.[15,16,17]

Conclusion

The abducens nerve palsy is the most common isolated ocular nerve palsy due to its neuroanatomy and the long intracranial course. Multiple etiologies are recognized based on the location of an abnormality, however the incidence of mass formation such as neoplastic lesion or cyst are rare. In the absence of risk factors or positive laboratory and clinical results, neuroimaging such as MRI scan can be a valuable diagnostic technique in determining the specific origin of sixth nerve palsy.

Acknowledgment

This case report received no specific grant from any funding agency in the public, commercial, or non-profit sectors.

References

1. Lucio LL, Freddi TD, Ottaiano AC. The Abducens Nerve: Anatomy and Pathology. Seminars in Ultrasound, CT and MRI. 2022; 43(5):414-419. https://doi.org/10.1053/j.sult.2022.04.008

2. Nakamizo A, Matsuo S, Amano T. Abducens nerve schwannoma: a case report and literature review. World Neurosurgery. 2019;125:49-54. https://doi.org/10.1016/j.wneu.2019.01.123

3. Erdal Y, Gunes T, Emre U. Isolated abducens nerve palsy: Comparison of microvascular and other causes. Northern Clinics of Istanbul. 2022;9(4):353. https://doi.org/10.14744%2Fnci.2021.15483

4. Kim HJ, Kim HJ, Choi JY, Yang HK, Hwang JM, Kim JS. Etiologic Distribution of Isolated Abducens Nerve Palsy: Analysis of 807 Patients and Literature Review. European Journal of Neurology. 2023; 30(8):2471-2480. https://doi.org/10.1111/ene.15828

5. Elder C, Hainline C, Galetta SL, Balcer LJ, Rucker JC. Isolated abducens nerve palsy: update on evaluation and diagnosis. Current neurology and neuroscience reports. 2016; 16(8):1-7. https://doi.org/10.1007/s11910-016-0671-4

6. Patel SV, Mutyala S, Leske DA, Hodge DO, Holmes JM. Incidence, associations, and evaluation of sixth nerve palsy using a population-based method. Ophthalmology. 2004;111(2):369–75. https://doi.org/10.1016/j.ophtha.2003.05.024

7. Ezer H, Banerjee AD, Thakur JD, Nanda A. Dorello's canal for laymen: a Lego-like presentation. Journal of Neurological Surgery Part B: Skull Base. 2012; 73(3):183-9. https://doi.org/10.1055/s-0032-1311753

8. Tubbs RS, Radcliff V, Shoja MM, Naftel RP, Mortazavi MM, Zurada A, Loukas M, Gadol AA. Dorello canal revisited: an observation that potentially explains the frequency of abducens nerve injury after head injury. World Neurosurgery. 2012;77(1):119-21. https://doi.org/10.1016/j.wneu.2011.03.046

9. Wang JM, Edwards BA, Loukas M, Oskouian RJ, Tubbs RS. Supernumerary abducens nerves: a comprehensive review. World Neurosurgery. 2018;112:39-45. https://doi.org/10.1016/j.wneu.2017.11.052

10. Nguyen V, Reddy V, Varacallo M. Neuroanatomy, Cranial Nerve 6 (Abducens). In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2023 Jan.

11. Graham C, Mohseni M. Abducens Nerve Palsy. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2023 Jan.

12. Chan NC, Li VS, Cheng AC, Cheung TC, Lo SS, Chan CK. Diagnostic pitfall of progressive isolated abducens nerve palsy: a report of two cases. Hong Kong Journal of Ophthalmology. 2022; 29;26(1). https://orcid.org/0000-0002-6473-4578

13. Baybora H, Kaya F, Koçak İ, Karabela Y. MRI results of patients with acute isolated cranial nerve palsies. Turkish Neurosurgery. 2020.

14. Nair AG, Ambika S, Noronha VO, Gandhi RA. The diagnostic yield of neuroimaging in sixth nerve palsy-Sankara Nethralaya Abducens Palsy Study (SNAPS): Report 1. Indian journal of ophthalmology. 2014; 62(10):1008. https://doi.org/10.4103/0301-4738.146000

15. Toda M, Kosugi K, Ozawa H, Ogawa K, Yoshida K. Surgical Treatment of Cavernous Sinus Lesion in Patients with Nonfunctioning Pituitary Adenomas via the Endoscopic Endonasal Approach. J Neurol Surg B Skull Base. 2018;79(Suppl 4):S311-S315. https://doi.org/10.1055%2Fs-0038-1667123

16. Sabermoghadam A, Etezad Razavi M, Sharifi M, Kiarudi MY, Ghafarian S. A modified vertical muscle transposition for the treatment of large-angle esotropia due to sixth nerve palsy. Strabismus. 2018;26(3):145-149. https://doi.org/10.1080/09273972.2018.1492621

17. Rowe FJ, Hanna K, Evans JR, Noonan CP, Garcia-Finana M, Dodridge CS, Howard C, Jarvis KA, MacDiarmid SL, Maan T, North L, Rodgers H. Interventions for eye movement disorders due to acquired brain injury. Cochrane Database Syst Rev. 2018;3(3):CD011290. https://doi.org/10.1002/14651858.cd011290.pub2

A Systematic Review of Efficacy and Safety of Difelikefalin in Treating Pruritus in Hemodialysis Patients with Chronic Kidney Disease

Muljani Enggalhardjo[1], Gabriella Hilary Tumiwa[2], Yeshiza Khosasih[2]

[1] *Department of Dermato-Venereology, Faculty of Medicine, Pelita Harapan University, Karawaci, Tangerang, Banten, Indonesia*
[2] *Faculty of Medicine, Pelita Harapan University, Tangerang, Banten, Indonesia*

Citation: Enggalhardjo Muljani, Tumiwa Gabriella Hilary, Khosasih Yeshiza. A Systematic Review of Efficacy and Safety of Difelikefalin in Treating Pruritus in Hemodialysis Patients with Chronic Kidney Disease. Medicinus. 2024 February. 13(2): 126-133.
Keywords: Pruritus; Hemodialysis; Chronic Kidney Disease; Difelikefalin
Correspondance: **Muljani Enggalhardjo**
E-mail: muljani.enggalhardjo@lecturer.uph.edu
Online First: **February 2024**

Abstract

Background: Chronic Kidney Disease (CKD) is a type of kidney disease that gradual loss of kidney function over a period of months or years, usually more than 3 months. Uremic pruritus or chronic kidney disease-associated with CKD (CKD-aP) is a commoncomplication that experienced by CKD patients especially for patients undergoing haemodialysis and it will negatively impact quality of life, for example depression, poor sleep quality, and miss dialysis sessions.

Methods: Three online databases were used for the literature search: Science Direct, Embase, and PubMed. obtaining the information in January 2024. Using specific keywords, a comprehensive analysis of research articles was carried out. We examined the safety and effectiveness of difelikefalin in the management of pruritus in patients receiving hemodialysis who have chronic kidney disease.

Result: Six studies were evaluated that met the criteria for inclusion. The efficacy of difelikefalin in all studies was examined by using WI-NRS as assessment tools for the primary outcome, and for the secondary outcome, skindex-10 or skindex-16 scoring, the 5-D itch scale, and the itch MOS (Medical Outcome Study) sleep disturbance scale were used. From all studies, difelikefalin in various dosages and routes (oral and intravenous) improved pruritus reduction in hemodialysis patients with CKD over placebo. However, in the majority of cases, difelikefalin caused a higher chance of experiencing adverse events than in the placebo group.

Conclusions: All studies show a greater pruritus reduction in hemodialysis patients receiving therapy over placebo, with the optimal benefit-risk at 0.5 µg/kg of difelikefalin, despite unclear efficacy-dosage connections.

Introduction

Chronic Kidney Disease (CKD) is a type of kidney disease that causes gradual loss of kidney function over a period of months or years, usually more than 3 months. Uremic pruritus or chronic kidney disease-associated with CKD (CKD-aP) is a commoncomplication that is experienced by CKD patients especially for patients undergoing haemodialysis. Pruritus linked to CKD may be caused by abnormalities in endogenous opioid receptor activation.[1]

Targeting peripheral kappa opioids receptors (KOR), difelikefalin is the first medication to be specifically licensed for the treatment of moderate-severe CKD-aP in patients receiving hemodialysis in the USA and Europe. Up to 40% of hemodialysis patients reported being moderate-extremely bothered by itching in the Dialysis Outcomes and Practice Patterns Study (DOPPS). Difelikefalin may also enhance sleep andquality of life when it comes to itching. Pruritus related to CKD will negatively impact quality of life, for example depression, poor sleep quality, and miss dialysis sessions.[2]

For hemodialysis patients with chronic kidney disease, the medication's safety and efficacy were critical considerations. This study will assess numerous factors that can reduce pruritic symptoms in hemodialysis patients with chronic kidney disease (CKD), including dosage, routes, side events, etc. Many methods are available to analyze the results, such the skindex-10 or skindex-16 scoring, the 5-D itch scale, the itch MOS (Medical Outcome Study) the sleep disturbance scale, and the WI-NRS (Worst Itch Numeric Rating Scale).

The effectiveness and safety of difelikefalin from multiple clinical trials will be presented in this study. The investigation of the function of activated kappa opioid receptors in the regulation of CKD- related systemic itch during hemodialysis is another goal of this work. Apart from that, there arestill a few studies that have reviewed this matter systematically to enhance evidence-based medicine.[3]

Material and Methods

Data Sources and Search Strategy

For this systematic review, three online databases were searched in January 2024: PubMed, Embase, and Science Direct. The search turned up 99 papers (21 from PubMed, 22 from Embase, and 56 from Science Direct) when the terms pruritus patient on hemodialysis were combined with AND efficacy OR effectiveness and AND difelikefalin. When we limited our search to pruritus patient on hemodialysis, published in the past 10 years randomized clinical trials, human studies, English publications, and the efficacy and/or effectiveness and safety of difelikefalin, we discovered 21 studies (3 from PubMed, 13 from Embase, and 4 from Science Direct). Three reviewers (ME, YK, and GHT) read the entire text. We eliminated from this research any studies that are not publicly available, in the form of presentations or posters, and have been published more than once. Consequently, there are six studies in the final selection stage (3 from PubMed and 3 from Embase) to form this systematic review.

Quality Assessment

We followed the PRISMA statement (http://www.prisma-statement.org) when conducting this systematic review. The Cochrane Collaboration risk of bias assessment (http://methods.cochrane.org/bias/assessing-risk- bias-included-studies) was then used to methodically evaluate the trial quality. Following the collection of studies, a more thorough evaluation of each study's eligibility was carried out, as shown in Figure 1.

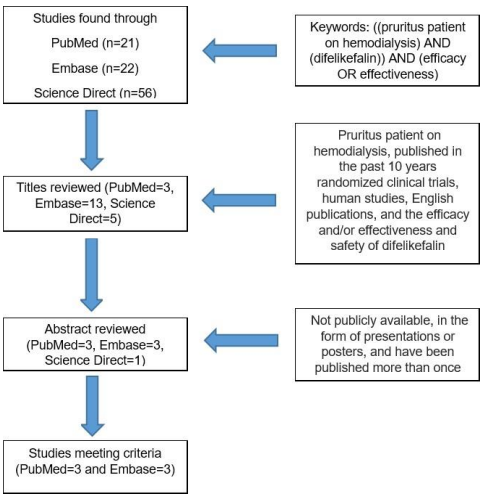

Figure 1. Flowchart of Study Collection

Table 1. Characteristics of Studies

Study & Location	Design	N	Study Criteria	Medications	Primary Outcome (Efficacy)	Secondary Outcome (Efficacy)	Adverse Event (Safety)
Fishbane et al.4 (2019) - US	Phase 3 double-blind, placebo controlled RCT with open label extension	189 and 188 subjects in difelikefalin andplacebo group respectively	Inclusion criteria: age ≥ 18 years (mean=58.2±11.2 difelikefalin group, 56.8±13.9 in placebo group), with end- stage kidney disease, had been undergoing hemodialysis at least 3 times per week for at least 3 months, had moderate-to- severe pruritus.	0.5 µg/kg difelikefalin or matched placebo intravenously three times a week for 12 weeks	≥ 3-Point improvement from baseline at week 12 in the weekly mean scores of the daily 24-hr WI-NRS scores: difelikefalin group > placebo group (49.1% vs 27.9%; RR, 1.65. 95% CI, 1.26-2.14; P<0.001)	Least-square mean change from baseline at week 12 in 5-D itch scale total scores: difelikefalin group > placebo group (-5.0±0.3 vs -3.7±0.3, P<0.001)	12-week double-blind intervention period: placebo group 62.2% = difelikefalin group 62.2% (overall AE)
					≥ 4-Point improvement from baseline at week 12 in the weekly mean scores of the daily 2-hr WI-NRS: difelikefalin group > placebo group (37.1 (28.3-46.9) vs 17.9 (12.1-25.8), RR, 1.92. 95% CI, 1.37-2.68; P<0.001)	Least-square mean change from baseline at week 12 in Skindex-10 scale total scores: difelikefalin group > placebo group (-17.2±1.3 vs -12.0±1.2, P<0.001)	2-week discontinuation period: placebo group 24.6% > difelikefalin group 19.9% (overall AE)
Yosipovitch et al.5 (2023) - US	Phase 2 double-blind, placebo-controlled RCT	69, 66, 67, 67 subjects in 0.25 mg, 0.5 mg, 1 mg, placebo difelikefalin groups respectively	Inclusion criteria: age ≥ 18 years (mean=65.7, 69, 67.5, 65,6, from defilekefalin 0.25 mg, 0.5 mg, 1 mg, and placebo group respectively), had moderate-severe renal impairment, had been receiving hemodialysis 3 times a week for ≥ 3 months, had moderate-to-severe pruritus.	0.25 mg, 0.5 mg, 1 mg, placebo difelikefalin orally once daily for 12 weeks (divided to 3 treatment groups and 1 placebo group)	≥ 3- and ≥ 4-Point improvement of WI-NRS at week 12: 14.4% < 31.6% (P-value:0.037) < 33% (P-value=0.027) < 38.6 (P=0.006) (placebo < 0.5 mg < 0.25 mg < 1 mg difelikefalin groups, rescpetively)	Least-square mean change from baseline at week 12 in 5-D itch total scores: -5.7 < - 6.2 (P value=0.515) < -6.8 (P value: 0.099) < -7.0 (P=0.070) in placebo, 0.25 mg, 0.5 mg, and 1 mg difelikefalin groups Rescpetively. Least-square mean change from baseline at week 12 in Skindex-10 total scores: -20.0 < -21.2 (P-value=0.580) < -22.1 (P-value:0.335) <-23.6 (P=0.116) in placebo, 0.25 mg, 0.5 mg, and 1 mg difelikefalin groups rescpetively	50.7% < 51.5% < 58.2% in placebo and 0.25 mg, 0.5 mg, and 1 mg difelikefalin groups rescpetively (overall AE) HD subjects > NDD-CKD subjects (TEAEs)
Narita et al.6 (2022) - Japan	Phase 2 double-blind, placebo-controlled RCT, multicenter, 4- arm, parallel-group	59, 53, 54, 59 subjects in 0.25 µg/kg, 0.5 µg/kg, 1 µg/kg, placebo difelikefalin groups respectively	Inclusion criteria: age ≥ 20 years (mean=64.5, SD=11.7), had ESKD and moderate-severe pruritus, had been receiving maintenance hemodialysis 3 times a week for at least 12 weeks, were nonresponse to systemic treatment (e.c.: antihistamines, and/or antiallergic drugs) and/or topical antipruritic moisturizers and a moderate or severe baseline Shiratori severity scores for 2 days or more in the 7 days preceding the start of treament.	0.25 µg/kg, 0.5 µg/kg, 1 µg/kg, placebo difelikefalin intravenously at the end of each hemodialysis session 3 times a week for 8 weeks (divided to 3 treatments groups and 1 placebo group)	≥ 3-Point improvement of WI-NRS at week 8: 50% < 53% < 57% < 60% (placebo < 0.25 µg/kg < 1 µg/kg < 0.5 µg/kg difelikefalin groups, rescpetively)	Adjusted weekly mean skindex-16 overall scores at week 8: -22.69. (SE=2.04) < -24.04 (SE=1.94) in placebo group < -24.25 (SE=1.96) < -27.79 (SE=2.05) in 1 µg/kg, placebo, 0.25 µg/kg, and 0.5 µg/kg difelikefalin groups respectively	67% < 72% <77%<85% in placebo 0.25 µg/kg, 0.5 µg/kg, 1 µg/kg difelikefalin groups, rescpetively (overall AE)
			Exclusion criteria: the pruritus was not associated with CKD, had liver cirrhosis as complication, history of phorotherapy, history of adverse events attributable to nalfurafine, and history of drug hypersensitivity to opioids.		≥ 4-Point improvement of WI-NRS at week 8: 34% < 36% < 43% < 51% (0.25 µg/kg < placebo < 1 µg/kg, < 0.5 µg/kg difelikefalin groups, rescpetively)	Adjusted weekly mean 5-D itch scale total scores at week 8: -5.8 (SE=0.3) < -6.6 (SE=0.3) < -6.5 (SE=0.4) < -6.8 (SE=0.3) in placebo,0.25 µg/kg, 0.5 µg/kg, and 1 µg/kg difelikefalin groups respectively	

Fishbane et al.7 (2020) - US	Phase 2 double-blind, placebo-controlled RCT	44, 41, 44, 45 subjects in 0.5 µg/kg, 1 µg/kg, 1.5 µg/kg, placebo difelikefalin groups respectively	Inclusion criteria: male or female adults, ≥ 18 years, had ESRD, had been receiving hemodialysis three times a week for at least three months prior to screening, had experienced persistent pruritus in the month preceding screening, with a weekly mean WI-NRS scores over the 7 days preceding randomization greater than 4 (ranging from 0 [no itching] to 10 [worst imaginable]).	0.5 µg/kg, 1 µg/kg , 1.5 µg/kg, placebo difelikefalin intravenously at the end of each hemodialysis session 3 times a week for 8 weeks (divided to 3 treatment groups and 1 placebo group)	≥ 3-Point improvement of WI-NRS at week 8: 29% < 59% < 64% < 67% (placebo < all combined < 0.5 µg/kg < 1.5 µg/kg difelikefalin groups, rescpetively)	Least-square mean change from baseline at week 8 in Skindex- 10 total scores: -8.2 (SEM=2.0) < -18.7 (SEM=2.0) < -15.5 (SEM=2.2) <-15.1 (SEM=2.3), 16.4 (SEM=1.3) in placebo, 0.5 µg/kg, 1 µg/kg, and 1.5 µg/kg, all combined difelikefalin groups rescpetively	42.2% < 70.7% < 77.3% < 77.5% < 84.1% in placebo, 1.0 µg/kg, 1.5 µg/kg, all combined, 0.5 µg/kg difelikefalin groups, respectively (overall AE)
					≥ 4-Point improvement of WI-NRS at week 8: 24% < 44% < 51% (placebo < all combined < 0.5 µg/kg difelikefalin groups, respectively)	Least-square mean change from baseline at week 8 in 5-D itch total scores: -2.8 (SEM=0.5) < -4.7 (SEM=0.6) < -5.3 (SEM=0.3) < -5.4 (SEM=0.6) < -5.7 (SEM=0.5) in placebo, 1.5 µg/kg, all combined, 1 µg/kg, 0.5 µg/kg difelikefalin groups rescpetively	
						Least-square mean change from baseline at week 8 in itch MOS sleep disturbance scores: -1.3 (SEM=3.1) < -6.9 (SEM=3.5) < -11.8 (SEM=2.0) < -13.8 (SEM=3.2) < -14.6 (SEM=3.4) in placebo, 1.5 µg/kg, all combined, 0.5 µg/kg, 1 µg/kg difelikefalin groups rescpetively	
Topf et al.8 (2022) - North America, Europe, and the Asia-Pacific region	Phase-3 double-blind, placebo controlled RCT with open label extension	426 and 425 subjects in difelikefalin and placebo groups respectively (these subjects were randomized in the pooled KALM-1 and KALM 2 studies	Inclusion criteria: ≥ 18 years (mean=58.3 and 59.1 in placebo and difelikefalin groups, respectively), had been treated by HD 3 times perweek for ≥ 3 months before screening. Exclusion criteria: had a scheduled kidney transplant during the study, had a concomitant disease or history of any medical condition, had been using new anti-itch treatments within 14 days before screening.	0.5 mcg/kg or placebo difelikefalin intravenously for 12 weeks	≥ 3-Point reduction in the weekly mean of daily WI-NRS at week 12 in KALM-1: 28.3% < 50.9% (placebo < difelikefalin groups, respectively)	≥ 15-Point improvements in Skindex-10 total scoress at week 12: 40.5% < 55% (placebo vs difelikefalin groups, respectively)	Overall AE: 3.8% < 6.3% (placebo vs difelikefalin groups, respectively)
					≥ 3-Point reduction in the weekly mean of daily WI-NRS at week 12 in KALM-2: 42.6% < 53.4% (placebo < difelikefalin groups, respectively)	≥ 15-Point improvements in 5-D itch total scoress at week 12: 42.3% < 52.1% (placebo vs difelikefalin groups, respectively)	
					≥ 4-Point reduction in the weekly mean of daily WI-NRS at week 12 in KALM-1: 18% < 38.4% (placebo < difelikefalin groups, respectively)	Least-square mean change from baseline at week 12 in Skindex-10 scores: -13.5 < -6.9 (placebo vs difelikefalin groups, respectively)	
					≥ 4-Point reduction in the weekly mean of daily WI-NRS at week 12 in KALM-2: 26.4% < 37.3% (placebo < difelikefalin groups, respectively)	Least-square mean change from baseline at week 12 in 5-D itch total scoress: -3.7 < -4.9 (placebo vs difelikefalin groups, respectively)	

Fishbane et al.9 (2022) - North America, Europe, and the Asia-Pacific region	Phase-3 double blind, placebo-controlled povotal phase 3, open-label studies	424, 424 subjects in difelikefalin and placebo, respectively	Inclusion criteria: ≥ 18 years (mean=58.4, 59, 58.3 in placebo, difelikefalin, and all-difelikefalin exposure cohort), had ESRD and were treated by HD 3 times per week for ≥ 3 months, had moderate-to-severe CKD-aP at baseline.	0.5 mcg/kg or placebo difelikefalin 3 times per week at the end of each dialysis session for 12 weeks	-	-	TEAE: 65.3% < 71.2% (placebo vs difelikefalin)
			Exclusion criteria: the use of new antipruritic medications after screening.				AEs leading to death: 1.2% > 0.7% (placebo vs difelikefalin groups, respectively)

Result

Literature Search

Seven citations' titles and abstracts from papers published in the previous ten years were examined. Because one of the studies used a clinical trial without a placebo, we had to eliminate it from our evaluation. Six studies in all fulfilled the search criteria, which included reporting the efficacy and safety of difelikefalin in treating pruritus related to chronic kidney disease in patients who had been receiving hemodialysis. Table 1 includes study design, number of patients, study criteria, medications, primary and secondary outcome.

Study Characteristics

The titles and abstracts of seven publications published during the last 10 years were analyzed. One study had to be excluded from our analysis as it employed a clinical trial without a control group. The search parameters were satisfied by six papers total, including those that reported on the safety and effectiveness of difelikefalin in treating pruritus associated with chronic kidney disease in hemodialysis patients. The primary and secondary outcomes, study criteria, number of patients, drugs, and trial design are all listed in Table 1. Numerous criteria were shared by all six studies. For example, those older than eighteen years, had end-stage renal disease or chronic kidney disease, for which hemodialysis was the treatment of choice three times a week for at least three months before the screening. The six studies were randomized controlled trials (RCTs) with a placebo. The major outcomes were assessed using the WI-NRS (Worst Itch Numeric Rating Scale) scores, while the secondary outcomes were assessed using the 5-D itch scale, itch MOS (Medical Outcome Study) sleep disturbance, skindex- 10, or skindex-16 scoring.

Administration of Difelikefalin

Difelikefalin and its placebo were given intravenously in five out of the six studies; as a result, only one research (Yosipovitch et al., 2023) delivered difelikefalin orally.[5] Oral difelikefalin was given once a day for 12 weeks by Yosipovitch et al. In the other studies, intravenous difelikefalin was administered three times a week, for eight to twelve weeks, following hemodialysis sessions.

Efficacy and Safety of Difelikefalin

Difelikefalin is a medication used to treat chronic renal disease-related pruritus. Difelikefalin (orally or intravenously) reduces pruritus in patients with chronic kidney disease. After two or three months of administering difelikefalin, ≥3- and/or 4-point improvements in WI-NRS as primary outcome showed that all of the trials demonstrated that difelikefalin produces superior results than a placebo group. The secondary outcome for those studies were evaluated using the 5-D itch scale, itch MOS sleep disturbance, skindex-10, or skindex-16, at two or three months after starting difelikefalin administration from baseline to examine the efficacy as well. All scores decreased more in difelikefalin groups

rather than in placebo groups, whether it was orally or intravenously administered. Some studies conducted the trial by giving one group the same dose, and some divided difelikefalin administration into several groups with different doses for each group (Fishbane et al., 2019; Yosipovitch et al., 2023; Narita et al., 2022).[4-6]

The safety of difelikefalin was evaluated based on AEs. According to Yosipovitch et al. (2023)[5], hemodialysis patients had a higher likelihood of treatment-emergent adverse events (TEAEs) or adverse events that started after the commencement of the trial drug compared to non-dialysis-dependent chronic kidney disease (NDD-CKD).

Dizziness and gastrointestinal AEs, which are the most prevalent TEAEs, were noted due to comorbidities and more severe illness in HD individuals. The majority of the studies showed overall AEs or TEAEs were found to be more common in difelikefalin groups than in placebo groups. However, Fishbane et al. (2022) showed AEs leading to death were found more in the placebo group than in the difelikefalin group (1.2% vs. 0.7%).[9]

Discussion

In order to determine the effectiveness and safety of difelikefalin in treating pruritus- related renal disease in hemodialysis patients, this article thoroughly reviewed the six most pertinent intervention trials. According to this review, the difelikefalin group showed a higher reduction in pruritus than the placebo group, as measured by the WI-NRS score, which was used to examine the primary outcome. For the secondary outcome, skindex-10 or skindex-16 scoring, the 5-D itch scale, the itch MOS (Medical Outcome Study), and the sleep disturbance scale were used in certain studies.

Three research conducted by Yosipovitch et al., Narita et al., Fishbane et al. (2020), compared the efficacy of difelikefalin starting from 0.25 µg/kg to 1.5 µg/kg. According to those three studies, the correlation between effectiveness and dosage has not reached a fixed answer.

Fishbane et al. (2020) and Narita et al. examined the efficacy based on WI-NRS scores after 8 weeks. However, from a study conducted by Fishbane et al., the highest ≥ 3-Point improvement of WI-NRS score after 8 weeks was found in the 0.5 µg/kg difelikefalin group. Meanwhile Narita et al., revealed the 1.5 µg/kg group gave the highest ≥3-Point improvement of WI-NRS score.

The lowest dose of difelikefalin that was given in all six research was 0.25 µg/kg. At the lowest dose, the difelikefalin group was still greater in giving improvement than placebo. The reason for this event is associated with the rate of drug clearance in hemodialysis patients. Hence, at all dosages, the exposure levels and difelikefalin efficacy were reached.[4-6]

Difelikefalin is a selective agonist of kappa opioid receptors that is selectively limited to the peripheral nervous system. It is thought to have a significant role in controlling chronic kidney disease associated with pruritus. Nevertheless, during the 12-week difelikefalin trial, there was no indication of abuse or any signs of physical dependence.[9] With one exception of a study by Fishbane et al. (2019), where the incidence in both trial groups was high and indicative of the vulnerable group of patients who present with serious other medical conditions, difelikefalin groups generally caused more adverse events than placebo groups.

Regarding safety, the majority of research revealed that AEs increased in frequency in a way that was dose dependent. Across all trials, nausea, vomiting, dizziness, diarrhea, and disturbances in gait were the most frequently reported adverse events. A dosage of 0.5 µg/kg of difelikefalin proved to have the most favorable benefit-risk profile. Difelikefalin's reaction at 0.25 µg/kg was clearly insufficient, as demonstrated by dose-response analysis, and it was safe to use up to 1.0 µg/kg.[6]

Conclusion

Many methods of evaluation showed that the group receiving therapy reduced pruritus more than the placebo group in hemodialysis patients with chronic renal disease, while the efficacy and dosage connection remain unresolved. Defelikefalin's safety varies according to dose. Consequently, it was discovered that 0.5 µg/kg of difelikefalin provided the optimal benefit-risk profile.

References

1. Shirazian S, Aina O, Park Y, Chowdhury N, Leger K, Hou L, et al. Chronic kidney disease-associated pruritus: Impact on quality of life and current management challenges. International Journal of Nephrology and Renovascular Disease. 2017; 10:11–26. https://doi.org/10.2147/ijnrd.s108045

2. Sukul N, Karaboyas A, Csomor PA, Schaufler T, Wen W, Menzaghi F, et al. Self-reported pruritus and clinical, dialysis-related, and patient-reported outcomes in hemodialysis patients. Kidney Medicine. 2021; 3(1). https://doi.org/10.1016/j.xkme.2020.08.011

3. Plewig N, Ofenloch R, Mettang T, Weisshaar E. The course of chronic itch in hemodialysis patients: Results of a 4- year follow- up study of GEHIS (German epidemiological hemodialysis itch study). Journal of the European Academy of Dermatology and Venereology. 2019; 33(7):1429–35. https://doi.org/10.1111/jdv.15483

4. Fishbane S, Jamal A, Munera C, Wen W, Menzaghi F. A Phase 3 Trial of Difelikefalin in Hemodialysis Patients with Pruritus. New England Journal of Medicine. 2020;382(3):222–32. https://doi.org/10.1056/nejmoa1912770

5. Yosipovitch G, Awad A, Spencer RH, Munera C, Menzaghi F. A phase 2 study of oral difelikefalin in subjects with chronic kidney disease and moderate-to-severe pruritus. Journal of the American Academy of Dermatology. 2023; 89(2):261-8. https://doi.org/10.1016/j.jaad.2023.03.051

6. Narita I, Tsubakihara Y, Uchiyama T, Okamura S, Oya N, Takahashi N, et al. Efficacy and Safety of Difelikefalin in Japanese Patients With Moderate to Severe Pruritus Receiving Hemodialysis. JAMA Network Open. 2022 ;5(5). https://doi.org/10.1001/jamanetworkopen.2022.10339

7. Fishbane S, Mathur V, Germain MJ, Shirazian S, Bhaduri S, Munera C, et al. Randomized Controlled Trial of Difelikefalin for Chronic Pruritus in Hemodialysis Patients. Kidney International Reports. 2020; 5(5):600–10. https://doi.org/10.1016/j.ekir.2020.01.006

8. Topf J, Wooldridge T, McCafferty K, Schömig M, Csiky B, Zwiech R, et al. Efficacy of Difelikefalin for the Treatment of Moderate to Severe Pruritus in Hemodialysis Patients: Pooled Analysis of KALM-1 and KALM-2 Phase 3 Studies. Kidney Medicine. 2022; 4(8):100512. https://doi.org/10.1016%2Fj.xkme.2022.100512

9. Fishbane S, Wen W, Munera C, Lin R, Bagal S, McCafferty K, et al. Safety and Tolerability of Difelikefalin for the Treatment of Moderate to Severe Pruritus in Hemodialysis Patients: Pooled Analysis From the Phase 3 Clinical Trial Program. Kidney Medicine. 2022; 4(8):100513. https://doi.org/10.1016%2Fj.xkme.2022.100513

RNAi-Based Therapy: Prospect as Cancer Treatment

Samuel E. Soentoro[1*], Michael Timothy[1*], Juandy Jo[1,2]

[1] *Department of Biology, Faculty of Science and Technology, Pelita Harapan University, Karawaci, Tangerang, Banten, Indonesia*.
[2]*Mochtar Riady Institute for Nanotechnology, Karawaci, Tangerang, Banten, Indonesia*.

Citation: Soentoro Samuel, Timothy Michael, Jo Juandy. RNAi-Based Therapy: Prospect as Cancer Treatment. Medicinus. 2024 February. 13(2): 134-144.
Keywords: RNAi; Cancer; Delivery systems
Correspondence: Juandy Jo
E-mail: juandy.jo@uph.edu
Online First: February 2024

Abstract

Cancer is one of the deadliest form of diseases in humans, with the annual deaths ranging in the millions. Conventional treatments including chemotherapy, radiotherapy, or surgery has their limitations, including common off-target and non-specific effects. Ribonucleic acid interference (RNAi) offers a new strategy for treating cancer by silencing specific genes to prevent gene expression. This review highlights the application of RNAi-based approach in targeting cancer, discusses its potential advantages and limitations, summarizes the existing clinical trials and provides a greater understanding of RNAi-based therapy in cancer.

Introduction

Cancer is one of the leading causes of mortality worldwide, accounting for nearly 10 million deaths in 2020 (nearly one in six deaths), with the most common cancers being breast, lung, colon, rectum, and prostate cancers.[1] Globally, the incidences of cancer have been constantly rising and are expected to reach almost 22 million per year by 2030.[2] Conventional cancer treatment usually involves surgery, chemotherapy, radiotherapy or a combination of those three modalities. However, their limitations hinder those conventional treatments, including the non-specific and off-target effects. The challenge of managing difficulties in treating numerous types of cancer necessitates the need for developing new strategies to improve treatment outcomes.

Cancer treatment through ribonucleic acid interference (RNAi) is an emerging field, which has shown promising evidence. Its therapeutic use is mediated through non-coding RNAs (ncRNAs), such as small interfering RNAs (siRNAs) and microRNAs (miRNAs), that act as gene-specific silencer. Those ncRNAs will bind and activate the RNA-induced silencing complex (RISC), a multi-protein complex. Subsequently they will bind to target messenger RNAs (mRNAs) and will degrade and cleave target mRNAs before their translation becoming proteins. This mechanism will interfere gene expression, resulting in post-transcriptional gene silencing.[3]

One advantage of RNAi-based treatment is its precision and accuracy, hence able to target the expression of specific target genes.[4] This means that it could potentially circumvent the hurdles commonly experienced among patients receiving chemotherapies, such as the rapid development of drug resistance and the risk of systemic toxicity due to the lack of specificity.[5] However, despite its promising applications, an issue that needs to be addressed is the issue regarding the ease of degradability of such RNA molecules.[6] Thus, the current focus of RNAi treatment is to look for the proper delivery methods, which ensures the appropriate concentration of the RNA reaching the target sites, where it can exert its therapeutic effect.

In this review, the application of RNAi-based approach in targeting cancer will be discussed in detail. In particular, this review will discuss its potential advantages and limitations, summarize the existing number of clinical trials and overall provide a greater understanding of RNAi-based therapy in cancer.

Principles of RNAi

As shown in **Figure 1**, the ncRNA molecules (e.g., miRNA or siRNA), which are double-stranded RNAs, are cleaved into shorter fragments by an enzyme called Dicer in the cytoplasm. Once this occurs, the miRNAs or siRNAs are loaded into the RISC, which acts as the key intermediate in the whole process. Each of miRNA or siRNA is unwound and split into two single-stranded RNAs, named the passenger and the guide strands, respectively. While the passenger strand is subsequently cleaved by the Ago 2 protein and degraded, the guide strand is incorporated into the RISC. The RISC then binds to target mRNA (through the assistance of the guide strand), before it cleaves and degrades target mRNA, resulting in inhibition of the gene expression.[7]

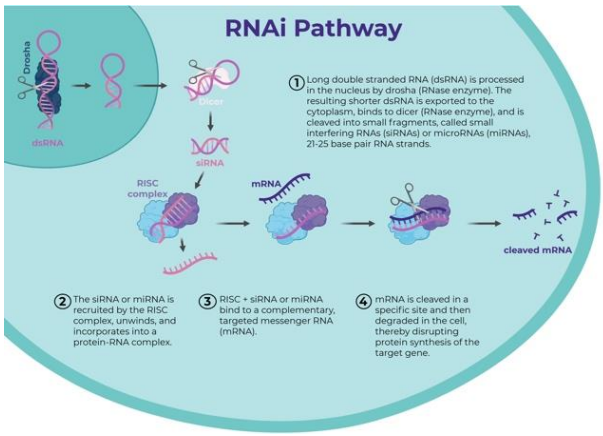

Figure 1. The action mechanism of RNAi. The picture was obtained from https://www.umassmed.edu/rti/biology/rna/how-rnai-works/

The use of RNAi as therapeutic agent

Due to its ability to target and silence specific genes, RNAi provides a novel opportunity to treat various diseases by targeting the expression of certain genes that are involved in the pathological processes.[8] Recent therapeutic approaches that targeting proteins, such as small-molecule inhibitors and immunotherapy, have been proven to be successful in several cases. However, even for protein-based drugs that are highly specific in nature (e.g., monoclonal antibodies), they are limited to targeting either freely circulating proteins or cell-surface receptors. In contrast, RNAi can overcome this limitation as RNAi can interfere with the expression of specific genes by blocking translation of their mRNA transcripts.[4]

RNAi-based therapy has been used in neurodegenerative diseases, cardiovascular diseases, infectious diseases and particularly cancer.[9] With the recent advancements in high-throughput sequencing technologies, the profiling of gene expression in cancer cells has facilitated the discovery of various dysregulated genes found in cancer cells. For this reason, RNAi therapy has been considered as an ideal strategy in silencing the expression of oncogenes.[8]

Strengths and challenges of RNAi

The application of RNAi-based therapy in the treatment of cancers offers several advantages. First, it can specifically target any gene within the cancerous cells.[10] This is crucial especially in the field of oncology, where many important therapeutic targets are currently undruggable using conventional drugs and immunotherapy.[11] Second, it can be easily synthesized and modified.[12] Third, it has good safety and efficacy profiles with low immunogenicity and it is highly specific with minimum off-target effects.[12] Indeed, RNAi-based therapy has shown promising *in vitro* tumor growth suppression and antiproliferative effect via the stat6 pathway and PLK1[13], angiogenesis suppression through inhibition of receptors such as VEGFs and VEGFR-1[14], as well as inhibition of tumor invasion and metastasis through chemokines CXCL8 and CXCL11.[15]

RNAi-based therapy has shown a great potential in terms of its therapeutic applications in several diseases including cancer.[9] However, there are issues that hinder RNAi-based therapy from being fully accepted into the clinical practice, such as its low stability and its delivery into cells.[16] For example, unprotected and unmodified RNA molecules are very easily degraded by endonucleases and can also trigger an immune response within the body.[16] Furthermore, since RNA-based therapy

relies on the cytoplasm entrance to exert its therapeutic effect, there are various challenges encountered during its delivery.[17]

Delivery methods of RNAi

As mentioned, one main limitation of RNAi-based therapy is that RNAs are extremely vulnerable to be degraded by ribonuclease presents in the body. Following their administration, RNA molecules are quickly degraded by the abundant sera ribonucleases, contributing to its short half-life *in vivo*. Even though double-stranded RNAs possess greater resistance towards ribonuclease degradation than single-stranded RNAs, the difference is insignificant.[18] Therefore, new methods are required to improve the resistance of ncRNA. This could be done by chemically modifying the RNA molecules to improve their stability. These chemical modifications can also help to solve other issues, such as minimizing immunogenicity and reducing off-target effects.[19] Currently, the common types of chemical modification which are studied in designing siRNA and miRNA are (i) locked and unlocked nucleic acids[20]; (ii) ribose 2′-OH group modification; and (iii) phosphorothioate modification.[19] Thus, depending on the desired function, different RNA modifications may be utilized.

Targeted delivery is another major obstacle in ensuring successfulness of RNAi-based therapy. Due to the inherently unstable nature of RNA, appropriate delivery vehicles are required that could protect RNA from degradation by ribonuclease. In addition, miRNA and siRNA have an intracellular site of action and thus require the entry of the RNA into the cytoplasm. However, due to their high molecular weight (~14–15 kDa) and polar nature, the overall properties of RNAi makes them poorly permeable across cellular membranes.[21] Therefore, novel delivery vehicles are required to deliver the ncRNA to the target site, protect ncRNA molecules from premature degradation by ribonuclease as well as to aid the cellular uptake of ncRNA into the cytoplasm, where it will exert its therapeutic effect.[22]

There have been numerous efforts put into designing and developing various delivery vehicles. Briefly, the viral vector and non-viral vector will be discussed as the RNA-delivery vehicles.

1. Viral Vectors

The usage of viral vectors, which encode either siRNAs or miRNAs, have been shown to trigger gene-silencing effects in several studies.[23] The main advantage of using viral vectors is their efficient ability to introduce the RNA-encoding genes into the cellular nucleus and ensure high expression of ncRNA. This facilitates the expression of multiple copies of ncRNA molecule from a single transcript, resulting in sustained gene silencing.[23] Several viruses have been utilized for this purpose, including lentiviruses[24], adenoviruses[25] as well as adeno-associated viruses (AAVs).[26]

However, there are several concerns in regards to the use of viral vectors, such as its high immunogenicity and the risk of viral genome insertion into the host's genome (i.e., insertional mutagenesis). These issues, along with the low packaging capacity and high production cost have limited the clinical applications of such viral vectors.[23] As a result, the use of non-viral vectors in delivering synthetic siRNAs and miRNAs is currently considered to be a better alternative due to their better safety profile and lower production cost.[27]

2. Non-viral vectors

Two main types of non-viral delivery systems are lipid-based and polymer-based systems

A. Lipid-based vector

A common lipid-based vector is liposomes, which are small spherical vesicle and are composed of a lipid membrane with a hydrophilic core. They protect the contents from degradation and have beneficial properties due to their low toxicity and immunogenicity.[28]

Furthermore, due to their hydrophilic core and hydrophobic lipid membrane, liposomes are able to fuse with the cellular membrane, which has a similar

characteristic. Following its fusion, the contents within the liposomes are released into the cytoplasm.[29] Liposomes can form a lipid complex with nucleic acid through electrostatic interaction, which aids in improving the overall stability and transfection efficiency. In this case, liposomes are given a positive charge to bind to the negatively charged nucleic acid content.[30] Cationic liposomes generally possess a greater encapsulation's efficiency when compared to their negatively charged counterparts. In addition, liposomes prolong the half-life of RNA in the blood by reducing its degradation.[30]

One modification to cationic liposomes is the addition of polyethylene glycol (PEG) to their surface to reduce the overall positive charge. This reduces the cytotoxicity and immunogenicity of liposomes as well as helps in prolonging the half-life of RNA in blood's circulation.[31] In the case of cancer, this modification has been shown to induce the uptake of lipid complexes into tumor cells as well.[9]

Besides liposomes, recent research has been focusing on the use of solid lipid nanoparticles (LNPs) as transporters. Solid LNPs address the issues found in liposomes, such as the stability and easier mass production due to the homogeneity of the particles.[32] Solid LNPs are composed of cationic lipids, cholesterol and polyethylene glycol (PEG), which still able to carry RNA and to protect it from degradation.[31] Solid LNPs generally have more complex internal structures as well as greater physical stability when compared to the less complex liposome.[32] An example of the usage is Patisiran, which is an siRNA-based therapy used to treat hereditary transthyretin-mediated amyloidosis.[33]

B. Polymer-based vector

This refers to the wide range of compounds, which are composed of monomers that interact with each other to form complex structures.[34] These materials commonly include synthetic polymers, such as poly(lactic-co-glycolic acid) (PLGA), polylactic acid (PLA), polyethylenimine (PEI), but can be composed of natural polymers, such as chitosan.[35] Similar to the lipid-based vector, these polymers are cationic structures, which can form complexes with negatively charged RNA through electrostatic interactions. Several advantages of such delivery systems are that in addition to the longer half-life, these polymers can be refined for delivering specific compounds through chemical modifications, hence allowing for a better control over the content's release.[25]

Chitosan is an example of a widely used and naturally derived vector. It is a polysaccharide composed of β-(1–4)-linked d-glucosamine and N-acetyl-d-glucosamine monomers. Due to its positive charge, chitosan and several of its derivatives have been extensively studied regarding its application as a vector for the delivery of both RNA and DNA.[36] Chitosan/siRNA nanoplex formulations have been studied for the delivery of PDGF-D and PDGFR-β–specific siRNA, efficiently reducing proliferation and invasion of breast cancer cells.[37] Oligochitosan nanoparticles have also been utilized in delivering siRNA against myeloid leukemia 1 (MCL1).[38]

PEI is one of the most used synthetic cationic polymers for delivering siRNA and miRNA, with both straight and branched forms in various molecular weights. PEI has high cationic charge density, which is an advantageous property as it can form small and compact complexes with nucleic acids.[39] It has also shown promising evidence of delivering siRNA to silence target gene expression.[40] Complexes composed of PEI and HER-2 receptor-specific siRNA have been shown to produce gene-silencing and anti-tumor effects in mice.[41] However, the cytotoxic effect of PEI in its unmodified state limits its clinical application. Thus, PEI should be combined with other polymers, such as hyaluronic acid, chitosan and PEG, in order to reduce its cytotoxicity and improve its siRNA delivery capabilities.[42]

RNAi-based therapy in cancer

Application of RNAi in cancer is mainly seen through the inhibition of tumor anti-apoptosis genes, inhibition of angiogenesis-related factors, inhibition of

oncogenes, as well as reduction of tumor drug resistance and other hallmark traits of cancer.

1. RNAi in Breast Cancer

Breast cancer is one of the most common forms of cancer, with 1 in 7 women being diagnosed in their lifetime.[53] Breast cancer can be divided into three subtypes based on the expression of hormone receptors: (i) hormone receptor positive (estrogen receptor positive and/or progesterone receptor positive), (ii) human epidermal growth factor receptor 2/HER2-enriched (with estrogen receptor negative and progesterone receptor negative), and (iii) triple negative (estrogen receptor negative, progesterone receptor negative and HER2 negative).[43] Accounting for 15-25% of invasive breast cancer, the HER2-enriched subtype has the worst prognosis among all subtypes. Although Trastuzumab, a HER2-target treatment based on monoclonal antibody, has subsequently improved patients' prognosis, drug resistance cases are still present and common among patients. The utilization of siRNA is postulated to be superior to antibodies or small molecule inhibitors because siRNA can intervene in an earlier stage of protein production, compared to antibodies or small molecule inhibitors, which bind to the protein to prevent its function.[44] Ngamcherdtrakul *et al*., 2015 showed that knockdown of HER2 using siRNA(siHER2) was able to increase numbers of apoptosis in HER2-enriched cancer cells *in vitro,* compared to HER2-targeted antibody (Trastuzumab) and a small molecule inhibitor (lapatinib).[45] Therefore, this method can potentially become an alternative treatment for HER2-enriched breast cancer.

2. RNAi in Pancreatic Cancer

Pancreatic ductal adenocarcinoma (PDAC) is amongst the deadliest types of cancers and is projected to become the second leading cause of cancer-related death.[46] Unlike other forms of solid cancer, effective PDAC treatment is unavailable thus far. The standard care of PDAC using chemotherapy treatments (FOLFIRINOX and gemcitabine/nab-paclitaxel) only yields a median overall survival of less than a year. A siRNA-based drug (Atu027) is under development to silence the expression of PKN3, a downstream effector of PI3 kinase signaling, in the vascular endothelium.[47,48] If it works, this treatment can potentially inhibit the tumor's metastatic spread.

3. RNAi in Lung Cancer

Lung cancer remains the deadliest form of the disease, affecting both men and women. In 2018, approximately 30,023 new cases and 26,095 deaths due to lung cancer were reported in Indonesia.[49] An involved gene in lung cancer is cyclooxygenase-2 (COX-2) which functions to regulate tumor progression, metastasis, and anti-tumor immunity. However, existent COX-2 inhibitors are ineffective to treat lung cancer. An alternate approach was designed to knockdown the delta-5-desaturase (D5D), which will limit the formation of arachidonic acid, a substrate for COX-2.[50] Preclinical studies have explored the possibility of using RNAi-based therapy for this purpose. Combining epithelial cell adhesion molecule (EpCAM) aptamers with D5D-specific siRNA and nanoparticles that binds D5D-specific siRNA showed a target-specific accumulation by suppressing the YAP1/TAZ axis.[51] Inhibited proliferation and induced apoptosis of lung cancer cells were indeed observed in lung cancer cell lines and murine models.[51] Thus, the utilization of nanoparticle-mediated RNAi-based therapy has the potential to overcome the constraints of conventional chemotherapy by selectively silencing the oncogenes and multi-drug resistant genes as well as minimizing the adverse risk towards healthy cells.[52]

Table 1. Clinical trials of RNAi-based therapy for cancers.

Drug Name	Clinical Phase	Condition	Targets	NCT ID	References
Rintatolimod	Approved in 2017	Chronic fatigue syndrome (CFS)	TLR3	NCT00215813	[54]
Lefitolimod (MGN1703)	Phase III	Metastatic Colorectal Cancer	TLR9	NCT02077868	[55]
STP705	Phase II	Basal cell carcinoma; Cutaneous squamous cell carcinoma; Hepatocellular carcinoma	TGF-β1 & COX-2	NCT04669808 & NCT04293679	[56]
Atu027	Phase II	Solid carcinoma; Pancreatic ductal carcinoma	PKN3	NCT01808638	[57]
siG12D LODER	Phase I	Pancreatic cancer; Pancreatic ductal adenocarcinoma	KRAS G12D	NCT01676259	[58]
APN401	Phase I	Pancreatic cancer; Colorectal cancer; Metastatic melanoma	Cbl-b	NCT03087591	[59]
TargomiRs	Phase I	Malignant pleural mesothelioma; Non-small cell lung cancer	miR-16	NCT02369198	[60]
DCR-BCAT	Pre-clinical	Hepatocellular cancer; Colorectal cancer	CTNNB1	N/A	[61]

Clinical trials of RNAi-based therapy for cancer

RNAi-based therapy has made remarkable progress in recent years, with more and more RNAi-based-drugs entering the market with each passing year. There are three FDA approved siRNA-based-drugs for non-cancer diseases thus far. Onpattro® (Patisiran) was approved in 2018 for treatment of polyneuropathy. Givlaari® (Givosiran) targeting mRNA of ALAS1 was approved in 2019 for treating acute hepatic porphyria (AHP). Oxlumo® (Lumasiran) targeting the HAO1mRNA as the treatment of primary hyperoxaluria type 1 was approved in 2020.[53]

Currently there is only one RNAi-based therapy in the market that has a potential to treat tumors. First launched in 2017 in Argentina, Rintatolimod, with a tradename of Ampligen® in the United States, is a mismatched double-stranded RNA molecule with immunomodulatory properties that acts as a Toll-Like Receptor 3 agonist.[62] Rintatolimod was able to reactivate the local immune response whilst stimulated the production of interferons and tumor necrosis factors.[63] Although this drug is licensed as a treatment for severe myalgic encephalomyelitis/chronic fatigue syndrome, it is currently tested in ongoing phase II/III clinical trials to evaluate its potential to treat stage II-IV HER2-enriched breast cancer, pancreatic cancer, renal cell carcinoma and other solid form tumors. Concurrently, *in vitro* studies suggested that the activation of TLR3 was able to induce apoptosis in lung cancer cell lines.[62]

Other RNAi-based therapy that has been determined to be safe in humans and are currently tested in advanced cancers include Atu027 for solid carcinoma and pancreatic ductal carcinoma; Lefitolimod for metastatic colorectal cancer; and TargomiRs for mesothelioma. The summary of currently tested RNAi-based therapy is shown in **Table 1**.

Conclusions

Cancer remains to be one of the deadliest and leading cause of human mortality. The malignant cell's ability to adapt and evade drug-induced cell death and immune responses render many medical treatments void. The search for a more precise and efficacious treatment is at the utmost importance. The silencing properties of RNAi offers potential in combating cancerous cells. As of now, many pharmaceutical companies are investigating the prospect of using RNAi-based therapy as cancer treatment.

References

1. World Health Organization (WHO). Fact Sheets: Cancer [Internet]. World Health Organization. World Health Organization. 2022. https://www.who.int/news-room/fact-sheets/detail/cancer

2. Bray F, Jemal A, Torre LA, Forman D, Vineis P. Long-term realism and cost-effectiveness: primary prevention in combating cancer and associated inequalities worldwide. J Natl Cancer Inst. 2015;107(12). https://doi.org/10.1093/jnci/djv273

3. Agrawal N, Dasaradhi PV, Mohmmed A, Malhotra P, Bhatnagar RK, Mukherjee SK. RNA interference: Biology, mechanism, and applications. Microbiol Mol Biol Rev. 2003; 67(4):657–85. https://doi.org/10.1128/mmbr.67.4.657-685.2003

4. Kim Y-K. RNA therapy: rich history, various applications and unlimited future prospects. Exp Mol Med. 2022;54(4):455–65. https://doi.org/10.1038%2Fs12276-022-00757-5

5. Svoboda P. Key mechanistic principles and considerations concerning RNA interference. Front Plant Sci. 2020;11.

6. Wang Y. Delivery systems for RNA interference therapy: Current technologies and limitations. Curr Gene Ther. 2020; 20(5):356–72. https://doi.org/10.2174/1566523220666201005110726

7. Neumeier J, Meister G. siRNA specificity: RNAi mechanisms and strategies to reduce off-target effects. Front Plant Sci. 2021;11. https://doi.org/10.3389/fpls.2020.526455

8. Chen X, Mangala LS, Rodriguez-Aguayo C, Kong X, Lopez-Berestein G, Sood AK. RNA interference-based therapy and its delivery systems. Cancer and Metastasis Rev. 2017;37(1):107–24. https://doi.org/10.1007/s10555-017-9717-6

9. Tian Z, Liang G, Cui K, Liang Y, Wang Q, Lv S, et al. Insight into the prospects for RNAi therapy of cancer. Front Pharmacol. 2021;12. https://doi.org/10.3389/fphar.2021.644718

10. Cao S, Lin C, Liang S, Tan CH, Er Saw P, Xu X. Enhancing chemotherapy by RNA interference. BIO Integration. 2020;1(2):64–81. http://dx.doi.org/10.15212/bioi-2020-0003

11. Roscigno G, Scognamiglio I, Ingenito F, Chianese RV, Palma F, Chan A, et al. Modulating the crosstalk between the tumor and the microenvironment using siRNA: A flexible strategy for breast cancer treatment. Cancers. 2020;12(12):3744. https://doi.org/10.3390%2Fcancers12123744

12. Jiang Y, Huo S, Hardie J, Liang X-J, Rotello VM. Progress and perspective of inorganic nanoparticle-based siRNA delivery systems. Expert Opin Drug Deliv. 2016;13(4):547–59. https://doi.org/10.1517/17425247.2016.1134486

13. Binnemars-Postma K, Bansal R, Storm G, Prakash J. Targeting the STAT6 pathway in tumor-associated macrophages reduces tumor growth and metastatic niche formation in breast cancer. FASEB J. 2018;32(2):969–78. https://doi.org/10.1096/fj.201700629r

14. Song Y, Tang C, Yin C. Combination antitumor immunotherapy with VEGF and PIGF siRNA via systemic delivery of multi-functionalized nanoparticles to tumor-associated macrophages and breast cancer cells. Biomaterials. 2018;185:117–32. https://doi.org/10.1016/j.biomaterials.2018.09.017

15. Hwang HJ, Lee Y-R, Kang D, Lee HC, Seo HR, Ryu J-K, et al. Endothelial cells under therapy-induced senescence secrete CXCL11, which increases aggressiveness of breast cancer cells. Cancer Lett. 2020; 490:100–10. https://doi.org/10.1016/j.canlet.2020.06.019

16. Vicentini FT, Borgheti-Cardoso LN, Depieri LV, Abelha TF, Petrilli R, Bentley MV. Delivery Systems and local administration routes for therapeutic siRNA. Pharm Res. 2013;30(4):915–31. https://doi.org/10.1007%2Fs11095-013-0971-1

17. Hattab D, Gazzali AM, Bakhtiar A. Clinical advances of siRNA-based nanotherapeutics for cancer treatment. Pharmaceutics. 2021;13(7):1009. https://doi.org/10.3390%2Fpharmaceutics13071009

18. Watts J, Deleavey G, Damha M. Chemically modified siRNA: Tools and applications. Drug Discov Today. 2008;13(19–20):842–55. https://doi.org/10.1016/j.drudis.2008.05.007

19. Layzer JM, McCafrey AP, Tanner AK, Huang Z, Kay MA, Sullenger BA. In vivo activity of nuclease-resistant siRNAs. RNA. 2004;10(5):766–71. https://doi.org/10.1261/rna.5239604

20. Damase TR, Sukhovershin R, Boada C, Taraballi F, Pettigrew RI, Cooke JP. The limitless future of RNA therapeutics. Front Bioeng Biotechnol. 2021;9. https://doi.org/10.3389/fbioe.2021.628137

21. Malburet C, Leclercq L, Cotte J-F, Thiebaud J, Bazin E, Garinot M, et al. Size and charge characterization of lipid nanoparticles for mRNA vaccines. Anal Chem. 2022;94(11):4677–85. https://doi.org/10.1021/acs.analchem.1c04778

22. Yin H, Kanasty RL, Eltoukhy AA, Vegas AJ, Dorkin JR, Anderson DG. Non-viral vectors for gene-based therapy. Nat Rev Genet. 2014;15(8):541–55. https://doi.org/10.1038/nrg3763

23. Couto LB, High KA. Viral vector-mediated RNA interference. Curr Opin Pharmacol. 2010;10(5):534–42. https://doi.org/10.1016/j.coph.2010.06.007

24. Kasar S, Salerno E, Yuan Y, Underbayev C, Vollenweider D, Laurindo MF, et al. Systemic in vivo lentiviral delivery of mir-15a/16 reduces malignancy in the NZB de novo mouse model of chronic lymphocytic leukemia. Genes & Immun. 2011;13(2):109–19. https://doi.org/10.1038/gene.2011.58

25. Lou W, Chen Q, Ma L, Liu J, Yang Z, Shen J, et al. Oncolytic adenovirus co-expressing miRNA-34a and IL-24 induces superior antitumor activity in experimental tumor model. J Mol Med. 2013;91(6):715–25. https://doi.org/10.1007/s00109-012-0985-x

26. Borel F, Kay MA, Mueller C. Recombinant AAV as a platform for translating the therapeutic potential of RNA interference. Mol Ther. 2014;22(4):692–701. https://doi.org/10.1038/mt.2013.285

27. Lam JK, Chow MY, Zhang Y, Leung SW. siRNA versus miRNA as therapeutics for gene silencing. Mol Ther Nucleic Acids. 2015;4(9). https://doi.org/10.1038%2Fmtna.2015.23

28. Chen Y, Zhu X, Zhang X, Liu B, Huang L. Nanoparticles modified with tumor-targeting scFv deliver siRNA and miRNA for cancer therapy. Mol Ther. 2010;18(9):1650–6. https://doi.org/10.1038/mt.2010.136

29. Pattni BS, Chupin VV, Torchilin VP. New developments in liposomal drug delivery. Chem Rev. 2015;115(19):10938–66. https://doi.org/10.1021/acs.chemrev.5b00046

30. Young SW, Stenzel M, Jia-Lin Y. Nanoparticle-siRNA: A potential cancer therapy? Crit Rev Oncol Hemato. 2016;98:159–69. https://doi.org/10.1016/j.critrevonc.2015.10.015

31. Tokatlian T, Segura T. siRNA applications in nanomedicine. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2010;2(3):305–15. https://doi.org/10.1002/wnan.81

32. Naseri N, Valizadeh H, Zakeri-Milani P. Solid lipid nanoparticles and nanostructured lipid carriers: Structure, preparation and application. Adv Pharm Bull. 2015;5(3):305–13. https://doi.org/10.15171%2Fapb.2015.043

33. Hoy SM. Patisiran: First global approval. Drugs. 2018;78(15):1625–31. https://doi.org/10.1007/s40265-018-0983-6

34. Ilarduya CT, Düzgüneş N, Sun Y. Gene delivery by lipoplexes and polyplexes. Eur J Pharm Sci. 2010;40(3):159-70. https://doi.org/10.1016/j.ejps.2010.03.019

35. Xin Y, Huang M, Guo WW, Huang Q, Zhang L Zhen, Jiang G. Nano-based delivery of RNAi in cancer therapy. Mol Cancer. 2017;16(1). https://doi.org/10.1186%2Fs12943-017-0683-y

36. Singh A, Trivedi P, Jain NK. Advances in siRNA delivery in cancer therapy. Artif Cells Nanomed Biotechnol. 2017;46(2):274–83. https://doi.org/10.1080/21691401.2017.1307210

37. Mao S, Sun W, Kissel T. Chitosan-based formulations for delivery of DNA and siRNA. Adv Drug Deliv Rev. 2010;62(1):12–27. https://doi.org/10.1016/j.addr.2009.08.004

38. Noh SM, Han SE, Shim G, Lee KE, Kim C-W, Han SS, et al. Tocopheryl oligochitosan-based self assembling oligomersomes for siRNA delivery. Biomaterials. 2011;32(3):849–57. https://doi.org/10.1016/j.biomaterials.2010.09.027

39. Şalva E, Özbaş S, Alan S, Özkan N, Ekentok-Atıcı C, Kabasakal L, et al. Combination therapy with Chitosan/siRNA nanoplexes targeting PDGF-D and pdgfr-β reveals anticancer effect in breast cancer. J Gene Med. 2022;25(2). https://doi.org/10.1002/jgm.3465

40. Zhang S, Zhao B, Jiang H, Wang B, Ma B. Cationic lipids and polymers mediated vectors for delivery of siRNA. J Control Release. 2007;123(1):1–10. https://doi.org/10.1016/j.jconrel.2007.07.016

41. Urban-Klein B, Werth S, Abuharbeid S, Czubayko F, Aigner A. RNAi-mediated gene-targeting through systemic application of polyethylenimine (PEI)-complexed siRNA in vivo. Gene Ther. 2004;12(5):461–6. https://doi.org/10.1038/sj.gt.3302425

42. Kim Y-K, Minai-Tehrani A, Lee, Cho C-S, Cho M-H, Jiang H-L. Therapeutic efficiency of folated poly(ethylene glycol)-chitosan-graft-polyethylenimine-PDCD4 complexes in H-RAS12V mice with liver cancer. Int J Nanomedicine. 2013;8: 1489-98. https://doi.org/10.2147/ijn.s42949

43. Johnson KS, Conant EF, Soo MS. Molecular subtypes of breast cancer: A review for Breast Radiologists. J Breast Imaging. 2020;3(1):12–24. http://dx.doi.org/10.1093/jbi/wbaa110

44. Ngamcherdtrakul W, Yantasee W. siRNA Therapeutics for breast cancer: Recent efforts in targeting metastasis, drug resistance, and immune evasion. Transl Res. 2019;214:105–20. https://doi.org/10.1016/j.trsl.2019.08.005

45. Ngamcherdtrakul W, Morry J, Gu S, Castro DJ, Goodyear SM, Sangvanich T, et al. Cationic polymer modified mesoporous silica nanoparticles for targeted siRNA delivery to $HER2^{+}$ breast cancer. Adv Funct Mater. 2015;25(18):2646–59. https://doi.org/10.1002/adfm.201404629

46. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, et al. Estimating the global cancer incidence and mortality in 2018: Globocan sources and methods. Int J Cancer. 2018;144(8):1941–53. https://doi.org/10.1002/ijc.31937

47. Schultheis B, Strumberg D, Kuhlmann J, Wolf M, Link K, Seufferlein T, et al. Safety, efficacy and Pharcacokinetics of targeted therapy with the liposomal RNA interference therapeutic ATU027 combined with gemcitabine in patients with pancreatic adenocarcinoma. A randomized phase IB/IIA Study. Cancers. 2020;12(11):3130. https://doi.org/10.3390%2Fcancers12113130

48. Schultheis B, Strumberg D, Santel A, Vank C, Gebhardt F, Keil O, et al. First-in-human phase I study of the liposomal RNA interference therapeutic ATU027 in patients with advanced solid tumors. J Clin Oncol. 2014;32(36):4141–8. https://doi.org/10.1200/jco.2013.55.0376

49. Suraya A, Nowak D, Sulistomo AW, Icksan AG, Berger U, Syahruddin E, et al. Excess risk of lung cancer among agriculture and construction workers in Indonesia. Ann Glob Health. 2021;87(1):8. https://doi.org/10.5334%2Faogh.3155

50. Khan P, Siddiqui JA, Lakshmanan I, Ganti AK, Salgia R, Jain M, et al. RNA-based therapies: A cog in the wheel of lung cancer defense. Mol Cancer. 2021;20(1):54. https://doi.org/10.1186/s12943-021-01338-2

51. Pang L, Shah H, Wang H, Shu D, Qian SY, Sathish V. EPCAM-targeted 3WJ RNA nanoparticle harboring delta-5-desaturase siRNA inhibited lung tumor formation via DGLA peroxidation. Mol Ther Nucleic Acids. 2020;22:222–35. https://doi.org/10.1016/j.omtn.2020.08.024

52. Magalhães M, Alvarez-Lorenzo C, Concheiro A, Figueiras A, Santos AC, Veiga F. RNAi-based therapeutics for lung cancer: Biomarkers, micrornas, and nanocarriers. Expert Opin Drug Deliv. 2018;15(10):965–82. https://doi.org/10.1080/17425247.2018.1517744

53. Kara G, Calin GA, Ozpolat B. RNAi-based Therapeutics and tumor targeted delivery in cancer. Adv Drug Deliv Rev. 2022;182:114113. https://doi.org/10.1016/j.addr.2022.114113

54. Jiang Q, Wei H, Tian Z. Poly I:C enhances cycloheximide-induced apoptosis of tumor cells through TLR3 pathway. BMC cancer. 2008. 8:12. https://doi.org/10.1186/1471-2407-8-12

55. Cunningham D, Salazar R, Sobrero A, Ducreux MP, Van Cutsem E, Scheithauer W, et al. Lefitolimod vs Standard of Care (SOC) for patients with metastatic colorectal cancer (mcrc) responding to first-line standard treatment: Results from the Randomized Phase III IMPALA trial. Ann Onc. 2019;30:v868–9. https://doi.org/10.1093/annonc%2Fmdz394.022

56. Kuźbicki Ł, Brożyna AA. Expression of cyclooxygenase-2 in human epithelial skin lesions. Appl Immunohistochem Mol Morphol. 2021. 29(3):163-174. https://doi.org/10.1097/pai.0000000000000871

57. Schultheis B, Strumberg D, Kuhlmann J, Wolf M, Link K, Seufferlein T, et al. Safety, efficacy and pharmacokinetics of targeted therapy with the liposomal RNA interference therapeutic ATU027 combined with gemcitabine in patients with pancreatic adenocarcinoma. A randomized phase IB/IIA Study. Cancers. 2020;12(11):3130. https://doi.org/10.3390/cancers12113130

58. Golan T, Khvalevsky EZ, Hubert A, Gabai RM, Hen N, Segal A, et al. RNAi therapy targeting KRAS in combination with chemotherapy for locally advanced pancreatic cancer patients. Oncotarget. 2015;6(27):24560–70. https://doi.org/10.18632/oncotarget.4183

59. Wolf D, Baier G. IFNγ helps CBLB-deficient CD8+ T cells to put up resistance to Tregs. Cancer Immunol Res. 2022;10(4):370–370. https://doi.org/10.1158/2326-6066.cir-22-0080

60. Reid G, Pel ME, Kirschner MB, Cheng YY, Mugridge N, Weiss J, et al. Restoring expression of miR-16: A novel approach to therapy for malignant pleural mesothelioma. Ann Oncol. 2013;24(12):3128–35. https://doi.org/10.1093/annonc/mdt412

61. Ganesh S, Cyr W, Koser M, Chopda G, Chipumuro E, Siddiquee Z, et al. Abstract 3827: Preclinical characterization of DCR-BCAT as a component of combination therapy. Cancer Res. 2016;76(14_Supplement):3827–3827. http://dx.doi.org/10.1158/1538-7445.AM2016-3827

62. Bianchi F, Alexiadis S, Camisaschi C, Truini M, Centonze G, Milione M, et al. TLR3 expression induces apoptosis in human non-small-cell lung cancer. Int J Mol Sci. 2020;21(4):1440. https://doi.org/10.3390%2Fijms21041440

63. Mitchell WM. Efficacy of rintatolimod in the treatment of chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME). Expert Rev Clin Pharmacol. 2016;9(6):755–70. https://doi.org/10.1586/17512433.2016.1172960