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Awareness and Knowledge of Celiac Disease Among the General Population in Saudi Arabia

Khalid I. AlHussaini,[1] Bassam Abdulaziz Alhusaini,[2] Saad Abdullah Alzmamy,[2] Omar Abdulaziz Alfozan,[2] Abdulaziz Mohammed Alnayil,[2] Turki Sulaiman Algannas,[2] Turki Yazeed Alassaf,[2] Osamah A. Hakami[3]

[1]Department of Internal Medicine, College of Medicine, Imam Mohammad Ibn Saud Islamic University, Riyadh, Saudi Arabia

[2]Faculty of medicine, Imam Mohammad Ibn Saud Islamic University, Riyadh, Saudi Arabia

[3]Department of Internal Medicine, King Abdullah Medical City in Holy Capital (KAMC-HC), Makkah, Saudi Arabia

Abstract

Celiac disease (CD) is an immune-mediated systemic disorder affecting nutrient absorption triggered by both environmental (gluten) and genetic factors. This study aimed to determine the level of awareness and knowledge of CD among the general population of Saudi Arabia. A community-based online cross-sectional study was conducted between August and October 2022 to examine the awareness regarding signs, symptoms, complications, and treatment of celiac disease. Data were collected and analyzed from 1,675 participants distributed across the five regions in Saudi Arabia. Analysis shows that 65.1% of the participants were familiar with the symptoms of celiac disease, and 49.6% confirmed that symptoms, blood tests, and endoscopy are the best way to diagnose celiac disease. The mean awareness score for the study participants was 71.8 (SD 13.9), with 5.2% of the participants needed a better level of awareness. Having a family history of celiac disease was an important predictor of having a higher level of awareness of celiac disease ($p<0.001$). The general public in Saudi Arabia demonstrates a moderate to high level of awareness of CD, emphasizing the need for extensive efforts to maintain high awareness of CD among the general public.

Keywords: Awareness, celiac disease, general population, gluten sensitivity, Saudi Arabia

Introduction

Celiac disease (CD) is a systemic immune-mediated disease that impacts the small intestine and results in mucosal destruction, primarily affecting individuals with a hereditary predisposition. This disease is triggered by gluten, a protein obtained from wheat, barley, and rye.[1] Currently, the sole therapeutic approach entails the lifelong avoidance of gluten consumption. As a chronic condition, if untreated, it has the potential to result in increased rates of morbidity and mortality.[2] Based on recent statistical data, it can be observed that CD, or celiac disease, is a prevalent ailment that impacts around 1 in 100 individuals globally. The prevalence of CD is commonly observed among individuals from Europe, the Middle East, South Asia, Africa, and South America. Nevertheless, the CD is infrequent among individuals of East and Southeast Asian descent, African Americans, and sub-Saharan Africans who lack the HLA-DR3-DQ2 haplotype, which has been linked to a heightened susceptibility.[3]

While there have been reports on the frequency of CD in Saudi Arabia, the precise total prevalence remains unknown. The study aims to investigate the frequency of CD in several regions of Saudi Arabia by a modest descriptive study. In a study conducted by Aljebreen et al.,[4] a cohort of healthy teenage students from three distinct districts in Saudi Arabia was included. The authors reported the prevalence of CD in each region, with Madinah having a prevalence of 1.8%, Aseer having a prevalence of 2.1%, and Al-Qaseem having a prevalence of 3.2%. According to a recent study, the prevalence of CD among school-aged children in Riyadh, Saudi Arabia, was found to be 1.5%.[4]

Previous research has proposed a higher likelihood of experiencing CD outbreaks in nations

Corresponding Author:
Bassam Abdulaziz Alhusaini
Faculty of medicine, Imam Mohammad Ibn Saud Islamic University, Riyadh, Saudi Arabia
Email: Bam.h98s@gmail.com

characterized by dietary patterns abundant in gluten, such as Saudi Arabia.[5] According to a study, 17.5% and 51.6% of the participants knew CD and gluten sensitivity. Approximately 17.5% of the restaurants in the study offer gluten-free meal alternatives. Additionally, 14.7% of these establishments have established protocols to prepare gluten-free food. Furthermore, a small proportion of 7.1% prominently display signs or notifications indicating the availability of gluten-free items. Lastly, a significant majority of 50.8% of the surveyed restaurants expressed their intention to incorporate gluten-free options into their menus in the foreseeable future. Furthermore, a study revealed that most gluten-free alternatives, precisely 82.5%, had a higher price point.[6]

The Kingdom of Saudi Arabia has an inadequate level of public awareness of gluten-related health concerns and CD. Hence, the primary aim of this research was to assess the level of awareness and information about CD within the general population of Saudi Arabia. The primary objective of this study was to investigate the level of awareness among the general population of Saudi Arabia regarding the signs, symptoms, and consequences associated with celiac disease. Additionally, this study sought to assess the knowledge about treating CD and adopting a gluten-free diet. Furthermore, we aimed to identify the sources of information that individuals in Saudi Arabia rely on for their understanding of CD.

Methods

A community-based online cross-sectional study was conducted between August and October 2022 to examine the awareness regarding signs, symptoms, complications, and treatment of celiac disease. Saudi adults aged 18 years and older formed the study population. This study excluded those below 18 years of age, incomplete questionnaires, and non-Saudis.

A convenient sampling technique was applied in this study. The questionnaire link was distributed to the general public in Saudi Arabia via a Google Forms link through social media platforms (Facebook, Twitter, Snapchat, and Instagram), along with a cover letter attached to a consent form. Participation was voluntary, with the option to withdraw at any time. All responses were anonymous, with no tracking of e-mail addresses or any identifying information.

The questionnaire was developed based on an extensive literature review to conduct this study. The socio-demographic variables in the survey questionnaire for the present analysis included gender, age, region of residence, education level, marital status, occupational status, family history of celiac disease, and how the participants rate their knowledge of celiac disease. The second part was about participants' awareness of celiac disease. Participants' awareness was examined regarding signs, symptoms, and complications of celiac disease and knowledge regarding celiac treatment and gluten-free diet.

The awareness of the general population toward celiac disease has been assessed using a 29-item questionnaire. The questionnaire has two patterns; item 1 to 8 was assessed by identifying the correct answers and marked as 1, and the incorrect answers were as 0; items 9 to 29 were a 5-point Likert scale type of questionnaire ranging from "strongly disagree" coded with 1 to "strongly agree" coded with 5. The total awareness score has been calculated by adding all 29 items. A possible score ranging from 21 to 113 points has been generated; a higher score indicates a higher awareness of celiac disease. By using 50% and 75% as the cut-off points to determine the level of awareness, participants were categorized as having poor awareness if the score was below 50%, 50% to 75% were categorized as moderate awareness, and above 75% were categorized as good awareness levels. In addition, the participants were asked about celiac disease treatment methods and their information sources.

During the first piloting phase, the questionnaire was randomly distributed to different regions of Saudi Arabia among 40 participants to measure the internal consistency of the study. These participants did not contribute to the findings of the main study. Based on the pilot study, the questionnaire was modified according to our research objectives and revised to overcome all obstacles faced during this pilot study. The reliability of the study questionnaire was measured using a 21-item questionnaire with 5-point Likert scale categories ranging from "strongly agree," coded as 1, to "strongly disagree," coded as 5. The overall reliability analysis of the 21-item questionnaires has a Cronbach Alpha of 0.889 indicating a very good internal consistency.

The required sample size from each study population was 385 participants, based on a confidence interval of 95%, a standard deviation of 0.5, and a margin of error of 5%.

The ethical approval for this study was

obtained from the ethical research committee of the Institutional Review Board (IRB) of Imam Mohammed Ibn Saud Islamic University (IMSIU), Riyadh, Saudi Arabia, wherein they reviewed and approved this project (HAPO-01-R-0011; Project No.273/2022).

Data were analyzed using Statistical Package for Social Science Software (SPSS), version 28. Categorical variables were shown as numbers and percentages (%), while continuous variables were presented as mean and standard deviation. The normality test was carried out using the Shapiro-Wilk test and Kolmogorov-Smirnov test. The differences in the awareness score about the socio-demographic characteristics and the perceived knowledge rating about CD were examined using the student-t test and ANOVA as appropriate. Binary logistic regression analysis was applied to identify predictors of higher awareness. The dummy variable for the binary logistic regression analysis was identified using the mean awareness score for the study sample as the cut-off point. The statistical significance level was assigned as 5.0%.

Results

The data was collected and analyzed from 1675 participants distributed across the five regions in Saudi Arabia. Around half of the participants (52.1%) were aged 18-25 years. More than half of the participants (64.5%) were females. Around 29.0% of the participants were from the Western region. More than half of the participants (74.6%) reported holding bachelor's degrees and were single (59.8%). Around 40.7% of the participants were university students. Around 8.9% of the participants reported that they have a family history of celiac disease. Around 45.6% of the participants confirmed that they were not aware of celiac disease. For further details on the socio-demographic characteristics of the study participants, refer to Table 1.

Table 2 represents the participants' knowledge of and awareness of celiac disease. In general, 45.6% indicated that they were not aware of celiac disease. About 64.8% of participants knew about gluten, and 46.6% had the opinion that children and adults have a high risk of developing celiac disease. Around 65.1% were familiar with the symptoms of celiac disease. Around 49.6% of participants confirmed that symptoms, blood tests, and endoscopy are the best way to diagnose celiac disease. The majority of them were not aware of the complications of celiac disease (70.7%). However, 53.9% believed that complications of celiac disease can be prevented.

Table 3 presents participants' awareness of celiac disease symptoms, prevention, complications, and products. A significant number of respondents strongly agreed that celiac disease symptoms include abdominal pain, diarrhea, flatulence, respectively, at 18.4%, 18.8%, and 18.0%, Furthermore, 21.3% of respondents strongly agreed that non-celiac gluten sensitivity should avoid eating gluten. The knowledge of celiac disease treatment could have been better; only 13.7% were aware that celiac disease can be cured. Only 5.4% of respondents strongly agreed that gluten-free products were available on the market, and only 10% strongly agreed that gluten-free dishes were available on restaurant menus. The mean awareness score for the study participants was 71.8 (SD 13.9). Around 5.2% of the participants showed poor level of awareness, 78.3% showed a moderate level of awareness, and 16.5% showed a good level of awareness, Table 4.

According to the participants' knowledge, a gluten-free diet was the most preferred method of treatment for celiac disease (25.6%), followed by oral medications (7.9%).

Social media was found to be the most commonly used source of celiac disease information (33.4%), followed by healthcare providers (29.3%) and family and friends (19.9%).

Table 5 presents the mean awareness score stratified by socio-demographic characteristics. Older patients, those who have a higher level of education, widowed or single participants, university students, and those having a family history of celiac disease had higher awareness scores compared to others ($p<0.05$).

Binary logistic regression analysis identified that having a family history of celiac disease was an important predictor of having a higher level of awareness of celiac disease ($p<0.001$). On the other hand, older age (36 years and over), being married, and being unemployed or employed were factors that increased the likelihood of having a lower level of awareness of celiac disease ($p<0.05$; Table 6).

Discussion

Celiac disease is a chronic immune-mediated disease that is initiated by the use of gluten and associated prolamins. This disease impacts

Table 1 Participants' socio-demographic characteristics

Variable	n (%)
Age group	
18–25 years	873 (52.1%)
26–35 years	417 (24.9%)
36–45 years	210 (12.5%)
>45 years	175 (10.4%)
Gender	
Male	595 (35.5%)
Female	1080 (64.5%)
Region of residence	
Central Region	432 (25.8%)
Southern Region	318 (19.0%)
Eastern Region	302 (18.0%)
Northern Region	146 (08.7%)
Western Region	477 (28.5%)
Education level	
Secondary or below	298 (17.8%)
University	1249 (74.6%)
Postgraduate	128 (07.6%)
Marital status	
Single	1002 (59.8%)
Married	616 (36.8%)
Divorced	44 (02.6%)
Widowed	13 (0.80%)
Occupational status	
Student	682 (40.7%)
Employee (governmental)	363 (21.7%)
Employee (non-governmental)	199 (11.9%)
Unemployed	373 (22.3%)
Retired	58 (03.5%)
Family history of celiac disease	
Yes	149 (08.9%)
No	1526 (91.1%)
How would you rate your knowledge of celiac disease?	
Strongly aware	183 (10.9%)
Aware	285 (17.0%)
Neutral	140 (08.4%)
Little aware	304 (18.1%)
Not aware	763 (45.6%)

Table 2 Assessment of Participant's Awareness of Celiac Disease

Awareness Statement	n (%)
Knowledge about gluten	
Yes *	1085 (64.8%)
No	590 (35.2%)
In your opinion, who are the people at risk of developing celiac disease	
Children only	105 (06.3%)
Adults only	73 (04.4%)
Children and adults *	780 (46.6%)
I don't know	717 (42.8%)
How many people do you think are affected by celiac disease?	
1.5–3% *	359 (21.4%)
15–35%	428 (25.6%)
45–60%	106 (06.3%)
70–80%	11 (0.70%)
I don't know	771 (46.0%)
Are you familiar with the symptoms of celiac disease?	
Yes *	585 (34.9%)
No	1090 (65.1%)
What do you think is the best way to diagnose celiac disease	
Symptoms only	66 (03.9%)
Symptoms and blood test	321 (19.2%)
Symptoms, blood test and endoscopy *	831 (49.6%)
I don't know	457 (27.3%)
Do you think that complications of celiac disease can be prevented?	
Yes *	902 (53.9%)
No	136 (8.1%)
I don't know	637 (38.0%)
Are you aware of the complications of celiac disease?	
Yes *	490 (29.3%)
No	1185 (70.7%)
Are you familiar with celiac disease treatment methods?	
Yes *	509 (30.4%)
No	1166 (69.6%)

various physiological systems and exhibits symptoms that can differ among individuals, particularly those with a genetic predisposition. A previous systematic review has demonstrated that the prevalence of CD among children in Saudi Arabia varies between 1.5% and 18.5%.[7] Consequently, this study aims to assess the extent of awareness and knowledge regarding CD among the general population in Saudi Arabia.

The study's findings revealed that most participants, specifically 45.6%, reported a lack of awareness regarding CD. This percentage is comparable to the results obtained from a study conducted in Saudi Arabia, where 48.4% of the participants had prior knowledge of CD.[8] Similar proportions of individuals who had never heard of the disease were observed in studies conducted in Turkey and the United Kingdom.[9] The study's

Table 3 Participants' Awareness Of Celiac Disease Symptoms, Prevention, Complications, and Products

Variable	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
Do you think abdominal pain is a symptom of celiac disease?	2.6%	6.9%	39.5%	32.6%	18.4%
Do you think diarrhea is a symptom of celiac disease?	3.0%	7.2%	38.5%	32.5%	18.8%
Do you think flatulence is a symptom of celiac disease?	2.8%	9.3%	40.8%	29.1%	18.0%
Do you think being underweight is a symptom of celiac disease?	2.8%	9.9%	42.0%	26.1%	19.2%
Do you think iron deficiency anemia is a symptom of celiac disease?	3.4%	12.8%	48.8%	21.6%	13.4%
Do you think infertility is a symptom of celiac disease?	9.0%	27.2%	50.0%	9.1%	4.8%
Do you think miscarriage is a symptom of celiac disease?	7.6%	22.9%	52.8%	11.5%	5.1%
Do you think skin sensitivity is a symptom of celiac disease	4.5%	15.7%	45.9%	22.7%	11.2%
People with non-celiac gluten sensitivity are strictly prohibited from eating gluten.	3.0%	7.2%	37.4%	31.2%	21.3%
People with celiac disease are completely prohibited from eating gluten.	3.0%	5.3%	37.2%	26.9%	27.6%
People with wheat allergy are completely prohibited from eating gluten	3.6%	8.6%	40.8%	27.1%	19.9%
Do you think anemia is a complication of celiac disease?	3.5%	10.3%	49.7%	21.7%	14.9%
Do you think osteoporosis is a complication of celiac disease?	3.5%	12.7%	49.5%	20.5%	13.9%
Do you think malabsorption is a complication of celiac disease?	2.9%	6.4%	40.7%	28.3%	21.7%
Do you think small bowel cancer is a complication of celiac disease?	3.4%	9.8%	51.2%	23.3%	12.3%
Do you think miscarriage is a complication of celiac disease?	5.3%	17.1%	55.9%	14.6%	7.1%
Do you think infertility is a complication of celiac disease?	6.3%	19.0%	57.1%	10.9%	6.7%
Do you think nutritional, vitamin and mineral deficiencies are a complication of celiac disease?	2.9%	7.0%	40.1%	29.7%	20.2%
Celiac disease can be cured once and for all with a treatment plan	6.1%	13.0%	41.8%	25.4%	13.7%
Are gluten-free products readily available in the market?	7.4%	31.5%	32.4%	19.8%	9.0%
Are gluten-free dishes available on restaurant menus?	15.2%	36.8%	32.3%	10.3%	5.4%

findings indicate that around 64.8% of the participants knew gluten, whereas 72.4% were aware of gluten sensitivity, as reported in the study conducted in Saudi Arabia.[6] Furthermore, it is worth noting that a significant proportion (46.6%) of the individuals involved in the study maintain the belief that children and adults face an increased susceptibility to the development of CD. It is essential to acknowledge that CD can manifest in genetically predisposed children at any stage from 9 months old until adulthood.[10] Various environmental factors contribute to the

Table 4 The Mean Awareness Score For The Study Participants

Variable	n (%)
Total awareness score (mean ± SD)	71.8 ± 13.9
Level of awareness	
Poor	87 (5.2%)
Moderate	1312 (78.3%)
Good	276 (16.5%)

risk of CD, including infant feeding practices, early infections, gut microbiota, multiple instances of antibiotic usage during early life, and the quantity and timing of initial gluten introduction.[11]

The results of the study showed that the vast majority of participants (78.3%) had a moderate level of awareness, where the mean awareness score for the study participants was 71.8 (SD 13.9), and around 5.2% of the participants showed a poor level of awareness, and 16.5% showed a good level of awareness, unlike study in Kuwait, where the vast majority of the participants showed a low level of awareness.[12] Also, the study results found that around 65.1% were familiar with the symptoms of celiac disease, and around 49.6% of participants

Table 5 Awareness Score Stratified by Socio-Demographic Characteristics

Variable	Awareness Score Mean ± SD	P-value
Age group		
18–25 years	72.9 (12.8)	
26–35 years	72.5 (14.01)	0.001**
36–45 years	70.2 (12.7)	
>45 years	69.4 (10.9)	
Gender		
Male	72.7 (13.0)	0.158
Female	71.8 (13.0)	
Educational level		
Secondary or below	70.7 (14.7)	
University	72.2 (12.5)	0.020 **
Postgraduate	74.5 (13.2)	
Marital status		
Single	72.8 (12.8)	
Married	71.0 (13.0)	0.039*
Divorced	71.5 (16.1)	
Widowed	75.1 (14.0)	
Occupational status		
Student	73.4 (12.9)	
Employee (Governmental)	73.1 (13.1)	
Employee (Non-Governmental)	69.0 (12.2)	<0.001***
Unemployed	70.2 (13.3)	
Retired	72.9 (10.4)	
Family history of celiac disease		
Yes	83.6 (12.3)	<0.001 ***
No	71.0 (12.5)	

* Significant p<0.05 level; ** Significant p<0.01 level; *** Significant p<0.001 level

Table 6 Predictors of a Higher Level of Awareness

Variable	Odds Ratio of Having a Higher Level of Awareness	p-value
Age group		
18–25 years (Reference group)	1.00	
26–35 years	0.93 (0.74–1.18)	0.559
36–45 years	0.63 (0.46–0.86)	0.003**
>45 years	0.58 (0.41–0.81)	0.001**
Gender		
Female (Reference group)	1.00	
Male	0.92 (0.75–1.13)	0.426
Educational level		
Secondary or below (Reference group)	1.00	
University	1.18 (0.92–1.52)	0.200
Postgraduate	1.41 (0.93–2.14)	0.102
Marital status		
Single (Reference group)	1.00	
Married	0.72 (0.59–0.88)	0.001**
Divorced	0.92 (0.51–1.69)	0.798
Widowed	1.18 (0.39–3.54)	0.767
Occupational status		
Student (Reference group)	1.00	
Employee (Governmental)	0.77 (0.60–1.00)	0.046*
Employee (non-governmental)	0.54 (0.39–0.74)	<0.001***
Unemployed	0.65 (0.50–0.84)	<0.001***
Retired	0.74 (0.43–1.26)	0.263
Family history of celiac disease		
No (Reference group)	1.00	
Yes	8.16 (5.09–13.09)	<0.001***

* Significant p<0.05 level; ** Significant p<0.01 level; *** Significant p<0.001 level

confirmed that symptoms, blood tests, and endoscopy are the best way to diagnose celiac disease, indeed, as reported in various studies, celiac disease presents with a range of symptoms, which can include diarrhea, failure to gain weight, irritability, oral cavity issues, chronic abdominal pain, fatigue, headache, bloating, weight loss, anemia, skeletal pain, and neuropathy.[13-15] This is similar to our study findings, where a significant number of respondents strongly agreed that CD symptoms include abdominal pain, diarrhea, flatulence, being underweight, iron deficiency anemia, infertility, miscarriage, and skin sensitivity.

Furthermore, the study's findings indicate that a significant proportion of the study participants (70.7%) lacked awareness regarding the potential complications associated with CD. Additionally, approximately 53.9% of the participants believed these complications could be prevented. It is worth noting that CD has been linked to an elevated risk of autoimmune diseases and cancer.[16] Moreover, ingesting gluten damages the small intestine in individuals with CD.[17] However, early detection and diagnosis of CD can play a crucial role in preventing the development of complications [18]. Furthermore, adhering to a gluten-free diet has been shown to

offer protection against most complications and reduce the associated mortality [16].

In addition, a gluten-free diet emerged as the predominant therapeutic approach for managing CD, with a preference rate of 25.6%. Oral drugs were also utilized but to a lesser extent (7.9%). Undoubtedly, adhering to a gluten-free diet represents the most productive and secure non-pharmacological intervention for individuals with CD.[19] A gluten-free diet encompasses naturally gluten-free foods such as meats, fruits, and vegetables and gluten-free alternatives to wheat, rye, and barley-based products like bread, pasta, and cereal. These alternatives typically use maize, rice, or millet flour.[19] However, it is important to note that an imbalanced gluten-free diet can increase the risk of metabolic issues. This underscores the significance of nutritional counseling in preventing and treating these complications.[20] Additionally, patients must comprehend the necessity of lifelong adherence to the gluten-free diet.[21] Consequently, the sole available treatment for CD is a strict exclusion of gluten from the diet.[22] Nevertheless, oral enzyme supplementation has been developed to expedite the breakdown of proline-rich gluten in the gastrointestinal tract.

The association between socio-demographic variables and awareness of celiac disease is intricate and has several aspects. Typically, increased education and income levels are associated with greater awareness, maybe because of improved access to information and healthcare. Youth are typically more informed, likely due to enhanced public health initiatives and educational endeavors in recent times. Women may have greater awareness than men, potentially attributed to variances in healthcare-seeking behavior. Geographic location and cultural influences contribute to differing levels of awareness seen among different regions and ethnic groups. A family history of CD has been identified as a significant predictor of increased awareness of the condition. It is known that individuals who have first-degree relatives with celiac disease are at a higher risk of developing the disease.[23] Additionally, the involvement of parents and children in the treatment of CD and their attitudes towards adhering to a restrictive gluten-free diet are essential factors influencing compliance with the treatment.[24] Notably, this involvement enhances awareness regarding CD within families with a history of the condition. In contrast, advanced age (36 years and older), marital status, and employment status were identified as factors associated with a higher likelihood of having a lower level of awareness regarding CD. It is worth noting that celiac disease can manifest in older individuals, although with less frequent symptom presentation, resulting in delayed diagnosis. This underscores the necessity of enhancing knowledge and awareness among the elderly population regarding CD.[25] Furthermore, it underscores the importance of healthcare professionals being knowledgeable about CD in the elderly and the advantages of adhering to a strict gluten-free diet. Failure to recognize the onset of symptoms in adults, associated comorbidities, and the significance of serology testing may contribute to the underdiagnosis of CD.

The study's findings emphasize the importance of implementing public health awareness efforts that specifically target persons at risk, particularly those with a familial predisposition to the disease. Furthermore, it is imperative to enhance the training of healthcare professionals in the accurate diagnosis of CD, with a specific focus on older individuals. Additionally, it is crucial to emphasize the significance of adhering to a strict gluten-free diet and provide essential nutritional counseling to prevent metabolic complications. Improving awareness and facilitating access to appropriate care can enhance the quality of life for individuals with CD while simultaneously reducing the risk of complications.

This study has limitations. The cross-sectional study design restricted our ability to infer causality among the study variables and follow up with the study participants. Online survey studies are prone to generalisability bias as the study participants might not represent the study population. Therefore, this study's findings should be interpreted carefully.

In conclusion, public awareness of CD in Saudi Arabia varied from moderate to high. The study highlights the necessity for organized public educational campaigns to raise knowledge about managing CD. Consistent efforts are needed to maintain a heightened public awareness of CD.

References

1. Fasano A, Catassi C. Clinical practice. Celiac disease. N Engl J Med. 2012;367(25):2419–26. doi:10.1056/NEJMcp1113994
2. Gujral N, Freeman HJ, Thomson AB. Celiac disease: prevalence, diagnosis, pathogenesis and treatment. World J Gastroenterol. 2012;18(42):6036–59. doi:10.3748/wjg.

v18.i42.6036
3. Gweon TG, Lim CH, Byeon SW. [A case of celiac disease]. Korean J Gastroenterol. 2013;61(6):338–42. doi:10.4166/kjg.2013.61.6.338
4. Aljebreen AM, Almadi MA, Alhammad A, Al Faleh FZ. Seroprevalence of celiac disease among healthy adolescents in Saudi Arabia. World J Gastroenterol. 2013;19(15):2374–8. doi:10.3748/wjg.v19.i15.2374
5. El-Metwally A, Toivola P, AlAhmary K. The epidemiology of celiac disease in the general population and high-risk groups in Arab Countries: a systematic review. Biomed Res Int. 2020;2020:6865917. doi:10.1155/2020/6865917
6. Khafagy AA, Qari WK, Filimban SS, Bahalaq AM, Bulkhi AA. A Cross-sectional study of celiac disease awareness in the food industry in the Western Region of Saudi Arabia. Cureus. 2022;14(6):e25613. doi:10.7759/cureus.25613
7. Alasmari Y, AlgethamI R, Ali N. Prevalence of celiac disease and its associated risk factors among children in the Kingdom of Saudi Arabia: A systematic review. Indo Am J P Sci. 2018;5(11):11593–600.
8. Alhussain M. Awareness of Celiac Disease among the General Public in Saudi Arabia. Int. J. Celiac Dis. 2021;9:71–6. doi:10.12691/ijcd-9-2-4
9. Taşkın B, Savlak N. Public awareness, knowledge and sensitivity towards celiac disease and gluten-free diet is insufficient: a survey from Turkey. Food Sci. Technol. 2020;41. doi:10.1590/fst.07420
10. Madden J. Celiac disease: more common yet "atypical" than previously thought. J Pediatr Neonatal Care. 2016;4:1-6. doi:10.15406/jpnc.2016.04.00123
11. Hadjivassiliou M, Sanders DS, Grünewald RA, Woodroofe N, Boscolo S, Aeschlimann D. Gluten sensitivity: from gut to brain. Lancet Neurol. 2010;9(3):318–30. doi:10.1016/S1474-4422(09)70290-X
12. Qasem WA, Roumi AA, Al Mojil K, Sakijha H, AlMughamis N. Awareness of celiac disease among the public in Kuwait: a cross-sectional survey. BMC Res. Notes. 2023;16(1):133. doi:10.1186/s13104-023-06415-x
13. Aktas G. Establishment the diagnosis of celiac disease. J Coll Physicians Surg Pak. 2019;29(12):1234–5. doi:10.29271/jcpsp.2019.12.1234
14. Hybenová E, Štofirová J, Mikulajová A. Celiac disease and gluten-free diet. Potravinarstvo. 2013;7doi:10.5219/276
15. Muggiano F, Quaranta A, Giannantoni I. Major dental clinical pathological manifestation of celiac disease. WebmedCentral Oral Medicine 2013;4(11):WMC004448
16. Cosnes J, Nion-Larmurier I. [Complications of celiac disease]. Pathologie-biologie. 2013;61(2):e21–6. Les complications de la maladie cœliaque. doi:10.1016/j.patbio.2011.03.004
17. Fiorilo C. Editorial note on celiac disease. J. Clin. Gastroenterol. 2021;6(1):1.
18. Jabeen R. Complications of celiac disease [Internet]. Celiac disease-from the bench to the Clinic. IntechOpen; 2019. Available from: http://dx.doi.org/10.5772/intechopen.80465.
19. Zevallos V, Herencia I. The gluten-free diet. Encyclopedia of food grains. 2016;2;91–7. doi:10.1016/B978-0-12-394437-5.00086-3
20. Marciniak M, Szymczak-Tomczak A, Mahadea D, Eder P, Dobrowolska A, Krela-Kaźmierczak I. Multidimensional disadvantages of a gluten-free diet in celiac disease: a narrative review. Nutrients. 2021;13(2):643. doi:10.3390/nu13020643
21. Raymond N, Heap J, Case S. The gluten-free diet: an update for health professionals. Practical Gastroenterology. 2006;30:1-11.
22. Zingone F, Capone P, Ciacci C. Celiac disease: Alternatives to a gluten free diet. World J Gastrointest Pharmacol Ther. 2010;1(1):36–9. doi:10.4292/wjgpt.v1.i1.36
23. Lee A, Iskander J, Gupta N. What's hot in the red journal this month. Am J Gastroenterol. 2011;106:1407–9. doi:10.1038/ajg.2011.243
24. Bagri DD, Gupta D, Mathur P. Assessment of parents' and child's attitude as barrier to dietary compliance in celiac disease. IJCP. 2016;15:53–8. doi:10.9790/0853-1508065358
25. Lerner A, Matthias T. Increased knowledge and awareness of celiac disease will benefit the elderly. Int. J. Celiac Dis. 2015;3:112–4. doi:10.12691/ijcd-3-3-6

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Antioxidant and Antiaging Properties of Ethanolic Ripe Sesoot Fruit Extract

Sri Utami,[1] Insan Sosiawan,[1] Dewi Nurul Mustaqimah,[1] Endah Purnamasari,[1] Lilian Batubara,[1] Qomariyah Romadhiyani Sachrowardi,[1] Ndaru Andri Damayanti,[1] Aryenti Aryenti,[1] Intan Keumala Dewi,[1] Said Nafik,[2] Betharie Cendera Arrahmani,[3] Hanna Sari Widya Kusuma,[4] Wahyu Widowati,[5] Herry S. Utomo[6]

[1]Faculty of Medicine, YARSI University, Central Jakarta, Indonesia
[2]Directorate General of Intellectual Property, Ministry of Law and Human Rights, Republic of Indonesia
[3]Pharmaceutical Bioprocessing Engineering, Technical University Munich, Freising, Germany
[4]Biomolecular and Biomedical Research Center, Aretha Medika Utama, Bandung, Indonesia
[5]Faculty of Medicine, Maranatha Christian University, Bandung, Indonesia
[6]Louisiana State University (LSU) AgCenter, H. Rouse Caffey Rice Research Station Rayne, LA, USA

Abstract

Skin aging can be characterized by changes in skin, such as the appearance of wrinkles and loss of skin moisture. Some elements that might lead to cell damages and aging are free radicals through the increase of the activities of hyaluronidase and tyrosinase. Bioactive compounds found in sesoot (*Garcinia picrorhiza* Miq.) are known for their antioxidant properties, which may reduce excessive amounts of free radicals in the body and act as an antiaging agent by inhibiting hyaluronidase and tyrosinase activities. This study aimed to examine the antioxidant, anti-hyaluronidase, and anti-tyrosinase properties found in Ethanolic Extract Ripe Sesoot (EERS). The ethanol extract of ripe sesoot fruit obtained from LIPI, Bogor Botanical Garden was used for the antioxidant and antiaging assays conducted at PT Aretha Medika Utama in December 2016. The activity of antioxidants was measured as the Ferric Reducing Antioxidant Power (FRAP) of the EERS, while the antiaging assays were performed through the inhibition activity of hyaluronidase and tyrosinase. Results indicated that the EERS has a higher FRAP activity (17.58 µM Fe (II)/µg) than xanthone (2.54 µM Fe (II)/µg) at the highest concentration of sample of 5,000 µg/mL. The anti-hyaluronidase of the EERS exhibited lower activity (IC50 of 619.21±12.15 µg/mL) than xanthone (IC50 of 365.55±25.10 µg/mL) and the tyrosinase inhibitory assay demonstrated a lower activity of EERS (IC50 of 1060.68±12.81 µg/mL) compared to xanthone (IC50 of 218.33±9.73 µg/mL). To conclude, EERS shows antioxidant and antiaging properties.

Keywords: Antioxidant**,** *Garcinia picrorhiza***,** hyaluronidase, skin aging, tyrosinase

Introduction

Aging is a progressive physiological change in an organism that causes senescence. It happens naturally in the human body. Aging is defined as the result of genetic programming and accumulation of environmental damage.[1,2] Functional changes associated with aging of the skin can result in rough, dry skin, decreased sweat, sebaceous gland secretions, and increased skin pigmentation, which can then cause hyperpigmentation, hypomelanosis guttate, and other irregular pigmentation.[3]

Skin aging is also highly related to hyaluronidase and tyrosinase. Hyaluronidase is an enzyme known to degrade hyaluronic acid by reducing its viscosity. Loss of hyaluronic acid leads to wrinkles and a decline in skin moisture.[4] The enzyme tyrosinase regulates Mammal skin, eyes, and hair pigmentation. These enzymes are crucial for initial immunological responses, wound healing, and pigmentation.[5]

However, the mechanism of the aging process has yet to be agreed upon unanimously among all gerontologists. The proposal of Denham Harman, which states that "free radicals are related to the chemical reactions at the bases of aging," is beginning to be well accepted.[6]

Corresponding Author:
Sri Utami
Faculty of Medicine, YARSI University, Central Jakarta, Indonesia
Email: uutsuyono@yahoo.com

Prior research has suggested that accumulating endogenous damage from reactive oxygen species (ROS) accelerates skin senescence. Non-free radical species like hydrogen peroxide (H_2O_2), superoxide anion radicals (O_2^-), and hydroxyl radicals (OH) are only a few examples of the extremely reactive, oxygen-containing species that are classified as ROS.[6]

On the other hand, *Garcinia* is known for its abundant source of phytoconstituents. It has been reported that all species from *Garcinia* contain some amount of garcinol, including Garcinia picrorhiza. Garcinol possesses a molecular weight of 602 and molecular formula represented by $C_{38}H_{50}O_6$. Garcinol is also known as camboginol and is similar to curcumin in structure. This chemical compound has been known to have many biological activity capacities, such as antioxidants and antimicrobials. This compound was also shown to scavenge free radical 1,1-diphenyl-2-picrylhydrazyl better than α-tocopherol, a well-established antioxidant. On top of that, it has been reported that garcinol might repress DNA damage by scavenging hydroxy radicals.[7] Garcinia picrorhiza is a woody plant natural to the islands of Maluku and Sulawesi and raised in equatorial climates [8,9]. Studies examining sesoot (*Garcinia picrorhiza* Miq.)'s bioactivity potential are quite limited, specifically its properties in inhibiting skin aging enzymes. Based on these, Ethanolic Extract Ripe Sesoot (EERS) was evaluated to discover its potential as an antioxidant and antiaging agent.

Methods

The *Garcinia picrorhiza* Miq. fruits used in this research were gathered from LIPI, Botanical Garden, Bogor, West Java, Indonesia. By Botanical Garden's herbarium personnel, *G. picrorhiza* Miq. fruits were precisely identified. The sampling method employed in this study is purposive sampling. A total of 500 g of fruits were crushed and then extracted with 70% distilled ethanol by maceration. The ethanol would then be filtered, and waste would be remacerated three times every 24 hours. The extract was then produced by collecting and utilizing a rotary evaporator to condense the filtrate from ethanol maceration. The rotary evaporator was set to 50°C..In preparation for future assays, the paste-formed extract was preserved at -20°C.[10] The standard used for this research was xanthone (Sigma, X0626). Xanthone was used as the standard on account of strong antioxidant value. A previous study reported that xanthone possessed an IC_{50} value of 4.21 µg/mL [11]. The extract and xanthone were then made into several final concentrations for each assay.

Assays of antioxidant and antiaging were conducted at PT Aretha Medika Utama in December 2016. The FRAP assay employed in this study was adapted from Widowati et al.[12] Reagent for FRAP assay was composed by freshly combining 10 mL of acetate buffer 300 mM pH 3.6 with 2.5 mL of 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (Sigma 3682-35-7, USA) 10 mM dissolved in 10% HCl 40 mM. The solution mixture was stored in an incubator at 37°C for 10 minutes. A 96-well microplate, accommodating a maximum of 7.5 µL of samples, was combined with 142.5 µL of the FRAP reagent and subsequently incubated at 37^0C for 30 minutes. The absorbance measurement at 593 nm was done by a microplate reader (Thermo Scientific Multiscan GO). $FeSO_4$ values varying from 0.019 to 95 µg/mL were utilized as the guidelines for standard curve. Results data from the samples were displayed.in µM Fe(II)/µg extract. The FRAP procedure is grounded on an antioxidant's capability to decrease (electron transfer) Fe^{3+} to Fe^{2+} ions in the appearance of TPTZ (2,4,6-tris(2-pyridyl)-s-triazine), resulting in the development of a Fe^{2+}-TPTZ complex with a bright blue color [13].

Hyaluronidase inhibitory activities were assessed using methods from Kolayli et al.,[14] and Sigma Aldrich with modifications from Widowati et al.[15] As much as 25 µL samples with various concentrations (0 to 166.67 µg/mL) were combined with 100 µL hyaluronidase (2 – 4 U/mg) (Sigma H3506, USA), 77 mM sodium chloride, 100 µL buffer phosphate (200 mM, pH 7, 37°C), and 0.01% BSA. The mixture was cultivated for 10 minutes at 37°C. A total 100 µL of hyaluronic acid substrate (Sigma H5542, USA) containing 0.03% in. 300 mM. sodium phosphate (pH 5.35) was.added.to the previous solution and cultivated.at 37°C. for 45 minutes. Hyaluronic decomposition was inhibited using 100 µL acidic albumin acid that contained 24 mM sodium acetate, 0.1% BSA, and 79.mM acetate acid (pH 3.75). After incubating the combined solution for 10 minutes at room conditions, absorbance measurement took place at a wavelength of 600 nm.

Inhibitory.activity :

$$\left(1-\frac{c-s}{c}\right)x\,100\left(1-\frac{c-s}{c}\right)x\,100$$

The formula above calculates the inhibitory activity of hyaluronidase. The C represents the absorbance of solutions without hyaluronidase, and S represents sample solutions.

The tyrosinase inhibitory activity assay was examined employing a modified procedure by Sandhu et al.,[16] Tu et al.,[17] and Widowati et al.[15] The sample was first procured in a 96 well-plate before the addition of 20 µL tyrosinase enzyme from fungal organisms (125 U/mL in potassium phosphate buffer), 140 µL of 20 mM potassium phosphate buffer (pH 6.8), and a 20 µL sample with various concentrations. Following this, the mixture underwent incubation at ambient temperature for a duration of 15 minutes. 20 µL of 1.5 mM L-DOPA was supplemented to the solution after the initial incubation period, and then it was re-incubated at room temperature for 10 minutes. The tyrosinase inhibitory activity was evaluated by determining the amount of DOPA generated and quantified at a wavelength of 470 nm.

Tyrosinase.Inhibitory Activity (%):

$$\frac{A-B}{A} x\ 100 \frac{A-B}{A} x\ 100$$

The formula above represents the Tyrosinase Inhibitory Activity, where A denotes the absorbance of the control sample and B denotes the absorbance of the test sample.

Statistical analyses were performed using One-Way ANOVA followed by the Tukey HSD Post Hoc test, with significance set at P<0.05P <0.05P<0.05. The data are presented as the mean ± standard deviation. All analyses were conducted using IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA).

Results

The basic principle of FRAP is to measure the decreasing concentration of the ferroin analog that occurs within the mixture. Tripyridyltriazine's Fe^{3+} complex Fe $(TPTZ)^{3+}$ is converted to $Fe(TPTZ)^{2+}$ by antioxidants in an acidic environment, and the latter exhibits a vivid blue color, measured at 593 nm.[18] The result of the FRAP assay belonging to EERS and xanthone is shown in Figure 1.

The current study's findings demonstrate that FRAP activity is concentration-dependent, with greater concentrations leading to increased FRAP activity (Figure 1). EERS exhibits stronger FRAP activity than xanthone (17.58 ± 0.48 µM Fe (II)/µg) at the maximum concentration of 5000

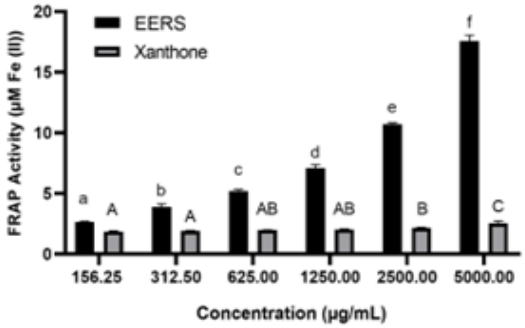

Figure 1 FRAP Activity of EERS and Xanthone

*Note: EERS and xanthone were diluted using DMSO to attain the final concentration of 156.25; 312.50; 625.00; 1250.00; 2500.00; 5000.00 µg/mL. The data is exhibited as means±standard deviations (SD). Varied letters (a, b, c, d, e, f) show significant contrasts among EERS concentrations and (A, AB, B, C) among xanthone concentrations, based on Tukey HSD test

µg/mL. This data indicates that EERS has lesser antioxidant activity in FRAP assay than xanthone (2.54 ± 0.18 µM Fe (II)/µg).

A particularly sensitive spectrophotometric technique was implemented to measure the inhibitory activity of hyaluronidase. The procedure is based on hyaluronidase acid precipitation with cetylpyridinium chloride, which is typically done for large-scale screening

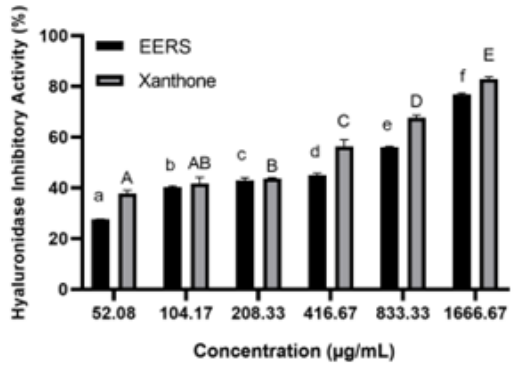

Figure 2 Hyaluronidase Inhibitory Activity of EERS and Xanthone

Note: EERS and xanthone were diluted using DMSO to reach the final concentration of 52.08; 104.17; 208.33; 416.67; 833.33; 1666.67 µg/mL. The data is exhibited as means±standard deviations (SD). Varied letters (a, b, c, d, e, f) show significant contrasts among EERS concentrations and (A, AB, B, C, D, E) among xanthone concentrations, based on Tukey HSD test

Table 1 IC_{50} Value Hyaluronidase Inhibitory Activity by EERS and Xanthone

Sample	Linear Equation	R^2	IC_{50} (µg/mL)
EERS	y = 0.0263x + 33.693	0.94	619.21 ± 12.15
Xanthone	y = 0.0277x + 39.856	0.95	365.55±25.10

Note: Linear equations, coefficient of regression (R^2) and IC_{50} of each sample were calculated. IC_{50} of each sample was calculated

Table 2 IC_{50} Value of Tyrosinase Inhibitory Activity by EERS and Xanthone

Sample	Linear Equation	r^2	IC_{50} (µg/mL)
EERS	y = 0.0449x + 2.4182	0.94	1060.68 ± 12.81
Xanthone	y = 0.1518x + 16.845	0.95	218.33 ± 9.73

Note: Linear equations, coefficient of regression (R^2) and IC_{50} of each sample were calculated. IC_{50} of each sample was calculated

of hyaluronidase inhibitors.[19] The result of this assay can be applied to assess antiaging activity of EERS and xanthone. The hyaluronidase inhibitory action of EERS and xanthone is depicted in Figure 2, whereas IC_{50} values are shown in Table 1.

Figure 2 proves the concentration dependence of hyaluronidase inhibitory action. At the. uppermost concentration of 1666.67 µg/mL, the hyaluronidase inhibitory activity of EERS (76.83±0.53%) is lower than that of xanthone (82.97±0.73%). In addition to that, the IC_{50} value of EERS (619.21±12.15 µg/mL) was also shown to be upper than that of xanthone (365.55±25.10 µg/mL) (Table 1). These two findings imply that, compared to xanthone, EERS has less hyaluronidase inhibitory action.

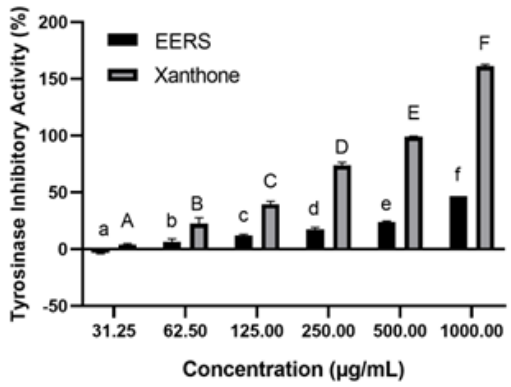

Figure 3 Tyrosinase Inhibitory Activity of EERS and Xanthone

Note: EERS and Xanthone were diluted with DMSO to reach the final concentration of 31.25; 62.50; 125.00; 250.00; 500.00; 1000.00 µg/mL. The data is exhibited as means±standard deviations (SD). Varied letters (a, b, c, d, e, f) show significant contrasts among EERS concentrations, and (A, B, C, D, E, F) between xanthone concentrations, based on Tukey HSD test

Tyrosinase inhibitory.activity was quantified to help determine EERS ability to detail the formation of melanin during melanogenesis. L-tyrosine (mono-phenol) has the potential to undergo hydrolysis into L-DOPA (di-phenol) catalyzed by tyrosinase, and subsequently, L-DOPA can be oxidized to form a dopaquinone (a quinone compound). Dopachrome is created when dopaquinone spontaneously reacts.[20]

The outcome of the assay has shown that tyrosinase inhibitory activity is concentration-dependent (Figure 3). At 1000 µg/mL, the highest concentration, EERS has lower inhibitory activity than xanthone, with a value of 46.55±0.09% as opposed to 161.20±1.36% from the latter. The IC_{50} value of EERS (1060.68±12.81 µg/mL) is higher than that of xanthone (218.33±9.73 µg/mL) (Table 2). This suggests that compared to xanthone, EERS has less antiaging action through tyrosinase inhibition.

Discussion

Sesoot is a species that belongs to the genus Garcinia, a well-known genus that possesses various pharmacological properties. According to reports, sesoot contains many bioactive compounds, such as xanthones, biflavonoidsa, and benzophenons.[21] *Garcinia* is usually used as a dietary supplement because of its antioxidant and antiaging activities.[22] In this study, EERS was examined for its potential as an antioxidant

through the FRAP assay and as an antiaging agent through the assays of the inhibitory activity of hyaluronidase and tyrosinase.

A FRAP assay was undertaken to measure antioxidant activities in reducing Fe^{3+} ions at low pH and causing the formation of the ferric-colored tripidilltriazin complex.[23] This finding. is consistent.with prior studies indicating that EERS has stronger antioxidant activity through H_2O_2 scavenging action when compared to xanthone. The current study found that EERS had higher antioxidant activity through FRAP inhibitory activity when compared to xanthone.[24] While α-mangostin had minimal activity, the extract and all fractions of *G. mangostana* had solid 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity.[23] In another study, EERS showed antioxidant activity by reducing ABTS free radicals.[24] Because of its capacity for scavenging, xanthone can also reduce the levels of DPPH and Fe^{3+}.[25]

Hyaluronan (HA, also known as hyaluronic acid) is a matrix-substantial tissue component and possesses a role in skin growth and development. Hyaluronic acid possesses the ability to keep the skin moisturized and well-hydrated. In the process of aging, the skin loses this critical component as a result of hyaluronidase activity. Hyaluronidase (Haases) can specifically degrade Hyaluronic Acid (HA), a large structural polysaccharide found exclusively within the Extracellular Matrix (ECM).[10] From this study, hyaluronidase inhibitory activity of EERS is lower than xanthone. However, other studies have shown that *G. indica* methanolic extract has a notable hyaluronidase inhibitory activity of 88.83%.

The tyrosinase enzyme is dependable for catalyzing the oxidation of L-DOPA to *o*-quinone with the help of L-tyrosine's (monophenol activity) hydroxylation. Both of these reactions help induce the production of the pigment melanin.[26] Microbial melanin is typically synthesized through either the DOPA (3,4-dihydroxyphenylalanine) pathway or the DHN (1,8-hihydroxynaphtalene) pathway. In the DOPA pathway, tyrosine is converted into levodopa, then dopaquinone, facilitated by enzymes like tyrosinase and laccase. Dopaquinone spontaneously polymerizes to form melanin.[27] In addition, many inhibitors of tyrosinase, such as kojic acid and arbutin, have been reported and evaluated as cosmetics and medicals to detain melanin production.[28,29] Based on research, the tyrosinase inhibitory activity of EERS is lower than that of xanthone. This study showed that EERS had more significant antioxidant activity than xanthone in the FRAP assay but less than xanthone in the hyaluronidase and tyrosinase inhibitory activity assay. However, this study is constrained by its limitations, as we have yet to characterize EERS with LC-MS/MS to discover its specific compounds that might be responsible for antioxidant and antiaging activity.

Previous research indicated that the methanol extract of *G. indica* exhibited hyaluronidase inhibitory activity within the concentration range of 500 to 750 µg/mL. Specifically, the methanol extract at a concentration of 1000 µg/mL demonstrated a hyaluronidase inhibitory activity of 90.5±0.01%. In contrast, the water fraction of *G. indica* did not show any significant hyaluronidase inhibition, even at very low concentrations.[30]

Based on the collected data, the EERS extract demonstrated superior activity in the FRAP antioxidant, anti-tyrosinase, and anti-hyaluronidase assays at the highest concentrations tested. The IC50 values for anti-hyaluronidase and anti-tyrosinase activities were 619.21±12.15 µg/mL and 1060.68±12.81 µg/mL, respectively. Additionally, the FRAP antioxidant test recorded a measurement of 17.58±0.48 µM Fe(II), surpassing the value observed for xanthone. These findings indicate that the EERS extract possesses notable antioxidant properties and exhibits effective inhibitory effects on enzymes associated with anti-aging mechanisms.

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References

1. Genovese L, Corbo A, Sibilla S. An insight into the changes in skin texture and properties following dietary intervention with a nutricosmeceutical containing a blend of collagen bioactive peptides and antioxidants. Skin Pharmacol Physiol. 2017;30(3):146–58. doi:10.1159/000464470
2. Miranda AF, Kenneth WM, Peter E, Howard

IM., Characteristics of the aging skin. Adv Wound Care. 2013;2(1):5–10. doi:10.1089/wound.2011.0356
3. Rizzi V, Gubitosa J, Fini P, Cosma P. Neurocosmetics in skincare-the fascinating world of skin-brain connection: A review to explore ingredients, commercial products for skin aging, and cosmetic regulation. Cosmetics. 2021;8(3):66.
4. Pintus F, Floris S, Fais A, Era B, Kumar A, Gatto G, et al. Hydroxy-3-Phenylcoumarins as multitarget compounds for skin aging diseases. synthesis, molecular docking and tyrosinase, elastase, collagenase and hyaluronidase inhibition, and sun protection factor. Molecules. 2022;27(20):6914. doi:10.3390/molecules27206914
5. D'Mello SAN, Finlay GJ, Baguley BC, Askarian-Amiri ME. Signaling pathways in melanogenesis.IntJMolSci.2016;17(7):1144. doi:10.3390/ijms17071144
6. Krumova K, Cosa G. Chapter 1: Overview of reactive oxygen species. Application in Biosciences and Nanosciences. 2016;1(2016):1–21.
7. Utami S, Sachrowardi QR, Damayanti NA, Wardhana A, Syarif I, Arrahman BC, et al. Antioxidants, anticollagenase and antielastase potentials of ethanolic extract of ripe sesoot (Garcinia picrorhiza Miq.) fruit as antiaging. J Herb Med Pharmacol. 2018;7(2):88–93.
8. Sukandar ER, Kaennakam S, Raab P, Nöst X, Rassamee K, Bauer R, et al. Cytotoxic and anti-inflammatory activities of dihydroisocoumarin and xanthone derivatives from *garcinia picrorhiza*. Molecules. 2021;26(21):6626. doi:10.3390/molecules26216626
9. Utami S, Laksono SP, Sachrowadi QR, Mustaqimah DN, Endrini S, Damayanti NA, Nafik S, Arrahmani BC, Kusuma HSW, Widowati W. In-vitro studies on antioxidant and antidiabetic potential of Sesoot (*Garcinia picrorhiza* Miq.) fruit ethanolic extract from Indonesia. Pharmaciana. 2021;11(2):273–82.
10. Widowati W, Fauziah N, Heddy H, Afni M, Afifah E, Kusuma HSW, et al. Antioxidant and antiaging assays of Oryza sativa extracts, vanilin and coumaric acid. J Nat Remed. 2016;16(3):88–99.
11. Cane HPCA, Saidi N, Mustanir M, Darusman D, Idroes R, Musman M. Evaluation of antibacterial and antioxidant activities of xanthone isolated from orophea corymbosa leaf. Rasayan J Chem. 2020;13(4):2215–22.
12. Widowati W, Widyanto RM, Husin W, Ratnawati H, Laksmitawati DR, Setiawan B, et al. Green tea extract protects endothelial progenitor cells from oxidative insult through reduction of intracellular reactive oxygen species activity. Iranian J Basic Med Sci. 2014;17(9):702–9.
13. Utami S, Damayanti, NA, Rahmah NA, Nafik S, Arrahmani, BC, Muthmainnah, AS, et al. In vitro studies on antioxidant potential of apple (*malus domestica*) fructus extract nanoparticle. J Natural Remedies. 202;23(4):1589–98.
14. Kolayli S, Huseyin S, Zehra C, Oktay Y, Kubra S. Honey shows potent inhibitory activity against the bovine testes hyaluronidase. J Enzyme Inhib Med Chem. 2016;31(4):599–602.
15. Widowati W, Rani AP, Hamzah RA, Arumwardana S, Afifah E, Kusuma HSW, et al. Antioxidant and antiaging assays of *Hibiscus sabdariffa* extract and its compounds. Nat Prod Sci. 2017;23(3):192–200.
16. Sandhu S, Bansal Y, Silakari O, Bansal G. Coumarin hybrids as novel therapeutic agents.BioorgMedChem.2014;22(15):3806–14. doi:10.1016/j.bmc.2014.05.032
17. Tu PTB, Tawata S. Antioxidant, antiaging and antimelanogenic propreties of the essential oil from two varieties of Alpinia zerumbet. Molecules. 2015;20(9):16723–40.
18. Utami S, Laksono SP, Sachrowardi QR, Mustaqimah DN, Endrini S, Damayanti NA, et al. In-vitro studies on antioxidant and antidiabetic potential of Sesoot (Garcinia picrorhiza Miq.) fruit ethanolic extract from Indonesia. Pharmaciana. 2021;11(2):273–82.
19. Eze FI, Uzor PF, Ikechukwu P, Obi BC, Osadebe PO. In vitro and in vivo models for anti-inflammation: An evaluative review. INNOSC Theranostics and Pharmacological Sciences. 2019;2(2):3–15.
20. Laksmiani NPL, Nugraha IPW. Depigmentation activity of secang (Caesalpinia Sappan L.) extract through tyrosinase, tyrosinase related protein-1 and dopachrome tautomerase inhibition. Biomed Pharmacol J. 2019;12(2):799–808.
21. Chantree P, Martviset P, Thongsepee N, Sangpairoj K, Sornchuer P. Anti-Inflammatory effect of garcinol extracted from garcinia dulcis via modulating NF-κB signaling pathway. Nutrients. 2023;15(3):575. doi:10.3390/nu15030575

22. Rizaldy D, Hartati R, Nadhifa T, Fidrianny I. Chemical compounds and pharmacological activities of mangosteen (Garcinia mangostana L.)-Update review. Biointerface Res Appl Chem. 2021;12(2021):2503–16.
23. Tjahjani S, Widowati W, Khiong K, Suhendra A, Tjokropranoto R. Antioxidant properties of Garcinia mangostana L (mangosteen) Rind. Procedia Chem. 2014;13(2014):198–203.
24. Utami S, Adityaningsari P, Sosiawan I, Endrini S, Sachrowardi QR, Laksono SP, et al. Antioxidants and anticholinesterase activities of the characterized ethanolic of ripe sesoot (Garcinia picrorhiza Miq.) fruit extract (EERS) and xanthone. Trad Med J. 2017;22(3):160–5.
25. Francik R, Szkaradek N, Oelaszczyk D, Maarona H. Antioxidant activity of xanthone derivatives. Acta Pol Pharm Drug Res. 2016;73(6):1505–9.
26. Kamal UZ, Sharique AA, Ayesha SA. Mushroom tyrosinase: recent prospects. Hindawi Publishing Corporation Biotechnology Research International. 2016;2016(2016):1–8.
27. Guo L, Li W, Gu Z, Wang L, Guo L, Ma S, et al. Recent advances and progress on melanin: from source to application. Int J Mol Sci. 2023;24(5):4360. doi:10.3390/ijms240543600
28. Chatatikun M, Chiabchalard A. Thai plants with high antioxidant levels, free radical scavenging activity, anti-tyrosinase and anti-collagenase activity. BMC Complement Altern Med. 2017;17(2017):487. doi:10.1186/s12906-017-1994-7
29. Guanhua W, Yue X, Wenjie S, Chuanling S. Lignin as a novel tyrosinase inhibitor: Effects of sources and isolation processes. ACS Sustainable Chem. 2018;6(7):9510–8.
30. Sahasrabudhe A, Deodhar M. Anti-hyaluronidase, anti-elastase activity of *Garcinia indica*. Int J Botany. 2010;6(3):299–303.

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Microbial Diversity and Antimicrobial Susceptibility from Wound Dehiscence Isolates in an Indonesian Tertiary Referral Hospital

Felicia Aviana, Ni Nengah Dwi Fatmawati
Department of Clinical Microbiology, Faculty of Medicine, Universitas Udayana/Prof. dr. I.G.N.G. Ngoerah Hospital, Denpasar, Bali, Indonesia

Abstract

Healthcare-associated infections (HAIs) remain a critical issue for public health in Indonesia, with wound dehiscence associated with surgical site infections (SSIs) being one of them. Globally, SSIs are known as the most common postoperative complications with a heightened prevalence, particularly in low to middle-income countries. With its retrospective and descriptive design, this study aimed to illustrate the microbial patterns identified in Prof. Dr. I.G.N.G. Ngoerah General Hospital from 12 January 2020 to 12 December 2022. The study includes all patients who underwent surgery and were subsequently diagnosed with wound dehiscence and SSIs. Specimens were collected from patients and submitted to the Microbiology Laboratory at the hospital above. Bacterial identification and susceptibility testing to antimicrobials were performed using the Vitek 2 Compact System (bioMerieux, Marcy l'Etoile, France). Patient information was sourced from medical records. Out of 172 samples, 151 (87.8%) yielded positive cultures. Among these, 151 (87.8%) were found to be positive. Gram-negative bacteria were found to be most prevalent, with *Escherichia coli* (20.2%) and *Pseudomonas aeruginosa* (19.6%) being the most frequently isolated bacteria. The bacteria isolated were mostly susceptible to amikacin (72.1%), followed closely by meropenem (71.4%). This information could contribute to the development of an empirical antibiotic therapy protocol for wound dehiscence or SSI cases in this local context.

Keywords: Antibiotics, bacterial infection, postoperative complication, surgical site infection

Introduction

Wound dehiscence stands as a formidable complication that surgeons are often wary of. It raises significant alarm due to its potential for evisceration, the possibility of needing subsequent interventions, the risk of recurrence, surgical wound infection, and the formation of an incisional hernia.[1] Wound dehiscence entails the separation of the borders of a surgical incision that has been closed on the skin, irrespective of whether the underlying tissues, organs, or implants are exposed or protruding. This separation could occur at one or multiple points or involve the entire length of the incision, affecting several or all layers of tissue. Not all dehisced incisions will show clinical signs or symptoms of infection.[2] As defined by the CDC, wound dehiscence could either be a superficial or deep tissue injury and may be linked to SSIs[3]. SSIs are now placing substantial financial strain on healthcare systems[1]. They are the most commonly observed type of infection in low- and middle-income nations, with incidence rates ranging from 1.2 to 23.6 per 100 surgical procedures and a combined incidence of 11.8%. In comparison, in developed countries, the rates of SSIs fluctuate between 1.2% and 5.2%.[4] SSIs account for around 20% of all healthcare-associated infections (HAIs), and a minimum of 5% of patients who undergo a surgical procedure develop an SSI.[5]

In 2021, the annual National and State Healthcare-Associated Infections (HAIs) Progress Report highlighted an increase in the SSI Standardized Infection Ratio (SIR) related to all combined National Healthcare Safety Network (NHSN) operative procedure categories, roughly 3%. However, minor changes were reported in SIR associated with the Surgical Care Improvement Project (SCIP) NHSN operative procedure categories compared to the

Corresponding Author:
Ni Nengah Dwi Fatmawati
Department of Clinical Microbiology, Faculty of Medicine, Universitas Udayana/Prof. dr. I.G.N.G. Ngoerah Hospital, Denpasar, Bali, Indonesia
Email: nnd.fatmawati@unud.ac.id

prior year.[6]

In hospital settings, antimicrobials are frequently employed as a preventative measure for all surgical procedures, with an inappropriateness rate of between 30% and 90%. Often, they are used at incorrect times, for extended periods, and with too broad a spectrum of coverage[7]. Overuse of prophylactic antimicrobials has the potential to modify the composition of normal flora or promote the spread of antimicrobial resistance.[7,8]

Hence, understanding bacterial etiology and the proper selection of antimicrobials is essential. This study sought to provide insights into bacterial identification and the pattern of antimicrobial susceptibility, particularly in patients with SSIs and wound dehiscence.

Methods

This descriptive study, using a retrospective approach, was carried out at Prof. Dr. I.G.N.G. Ngoerah General Hospital from 12 January 2020 through to 12 December 2022. Samples were gathered from patients suffering from wound dehiscence or SSIs and processed at the same hospital's Microbiology Laboratory. The Faculty of Medicine Udayana University Ethics Committee granted ethical approval for this study (No: 134/UN14.2.2.VII.14/LT/ 2023).

In total, 172 wound swabs, pus, and tissue samples were gathered from patients diagnosed with SSIs or wound dehiscence. Each specimen was transferred to the microbiology lab within 2 hours by placing the swab in Amies transport medium. The collected swabs were spread on sheep blood agar and MacConkey agar plates and then incubated at 37 degrees Celsius for a duration of 24 to 48 hours. Bacterial identification was conducted using the Vitek 2 Compact system (bioMerieux, Marcy l'Etoile, France).

Tests for antimicrobial susceptibility were also executed using the Vitek 2 Compact system (bioMerieux, Marcy l'Etoile, France). Bacterial suspension with a 0.5 McFarland standard was diluted to 1.5 × 107 CFU/ml in 0.45% saline solution. Testing was performed according to the manufacturer's instructions. The antimicrobial susceptibility tests were conducted for each bacterial species using susceptibility testing cards for *Staphylococcus spp., Enterococcus spp., and S. agalactiae* (AST-GP67 panel), for *S. pneumoniae, beta-hemolytic Streptococcus, and Viridans Streptococcus* (AST-ST03 panel), and a gram-negative (AST- GN93 panel). VITEK 2 results were read and analyzed automatically using the software provided by bioMerieux (version 9.02). For all the identification and antibiotic susceptibility test were performed, *Escherichia coli* (ATCC) 25922 and *Staphylococcus aureus* (ATCC) 29213 were used as reference strains for quality controls.

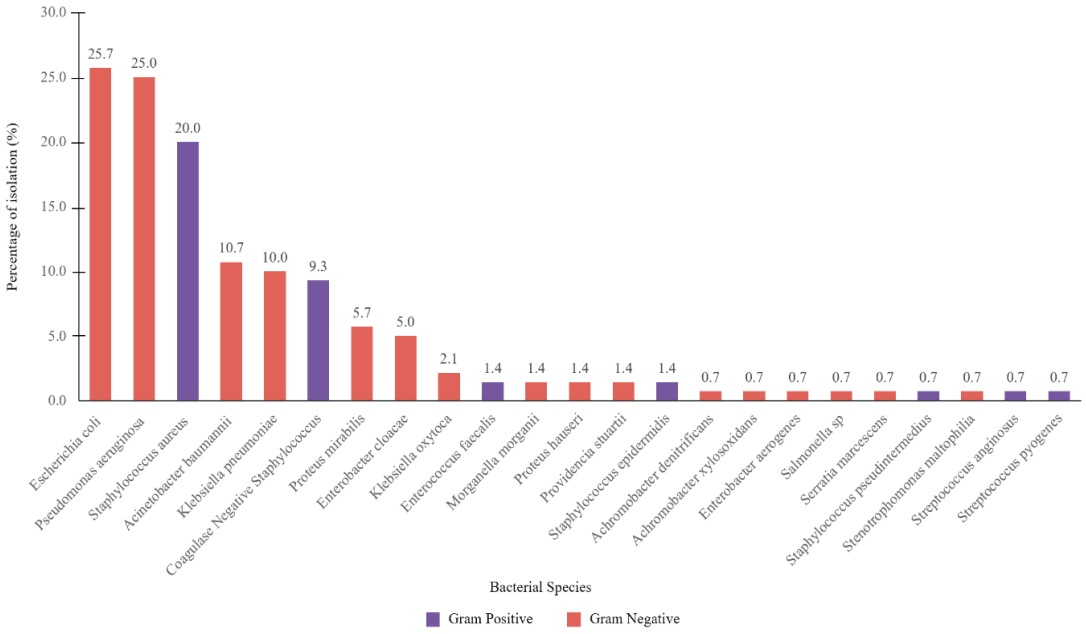

Figure 1 The Pattern of Isolated Bacteria from Wound Swabs, Pus, And Tissue Specimens in the Clinical Microbiology Laboratory, Prof. dr. I.G.N.G. Ngoerah Hospital

Table 1 Antibiotic Sensitivity Pattern Of Gram-Positive Isolates (n=29)

Antibiotics	Sensitivity n (%)
Linezolid	28 (96.6)
Tigecycline	28 (96.6)
Vancomycin	28 (96.6)
Erythromycin	25 (86.2)
Levofloxacin	25 (86.2)
Azithromycin	24 (82.8)
Ciprofloxacin	24 (82.8)
Clindamycin	24 (82.8)
Gentamycin	24 (82.8)
Moxifloxacin	24 (82.8)
Ampicillin/Sulbactam	20 (69.0)
Ceftriaxone	20 (69.0)
Trimethoprim/ Sulfamethoxazole	20 (69.0)
Cefepime	19 (65.5)
Cefuroxime	18 (62.1)
Piperacillin/Tazobactam	18 (62.1)
Tetracycline	18 (62.1)
Doxycycline	16 (55.2)
Amoxicillin/Clavulanate	15 (51.7)
Cefazolin	14 (48.3)
Amoxicillin	9 (31.0)
Ampicillin	2 (6.9)

Table 2 Antibiotic Sensitivity Pattern of Gram-Negative Isolates (n=111)

Antibiotics	Sensitivity n (%)
Amikacin	101 (91.0)
Meropenem	100 (90.1)
Piperacillin/Tazobactam	74 (66.7)
Gentamycin	67 (60.4)
Cefepime	61 (55.0)
Tigecycline	60 (54.1)
Ceftazidime	57 (51.4)
Ciprofloxacin	54 (48.6)
Aztreonam	45 (40.5)
Trimethoprim/ Sulfamethoxazole	35 (31.5)
Levofloxacin	34 (30.6)
Cefoperazone/Sulbactam	32 (28.8)
Ampicillin/Sulbactam	22 (19.8)
Ceftriaxone	18 (16.2)
Cefixime	12 (10.8)
Cefoperazone	12 (10.8)
Ampicillin	6 (5.4)
Cefuroxime	2 (1.8)

All the data gathered were analyzed using descriptive statistics on SPSS version 22. Results were expressed as frequencies and percentages and presented in figures and tables.

Results

The study's findings indicated that of the 172 samples, 87.8% (151/172) were positive for bacterial growth, with the remaining being negative. Single-bacterial growth was observed in 82.1% (124/151) of the positive samples, and multi-bacterial growth was detected in 17.8% (27/151). As a result, the total number of bacterial isolates amounted to 178. Of these isolates, 78.6% (140/178) were identified as pathogenic bacteria, while 21.3% (38/178) were classified as colonization bacteria. Of the 178 bacterial isolates, Gram-negative bacteria were the most prevalent, comprising 73% (130/178) of the isolates, whereas Gram-positive bacteria comprised 26.9% (48/178). The detailed breakdown of bacteria extracted from patient samples is depicted in Figure 1. Altogether, 23 distinct bacterial species were identified. The most frequently isolated bacterium was

Escherichia coli 20.2% (36/140), followed by *Pseudomonas aeruginosa* 19.6% (35/140) and *Staphylococcus aureus* 15.7% (28/140).

Regarding the 140 pathogenic bacterial isolates subjected to antimicrobial sensitivity testing, the antimicrobial susceptibility test results revealed that 72.1% of these isolates were susceptible to amikacin, closely followed by meropenem at 71.4%. The Gram-positive pathogenic bacteria showed the highest sensitivity towards linezolid, tigecycline, and vancomycin, each with a susceptibility rate of 96.6% (Table 1). In contrast, the Gram-negative pathogenic bacteria exhibited the greatest sensitivity to amikacin (91%) and meropenem (90.1%), as outlined in Table 2.

Discussion

In countries with low to medium income, surgical site infections (SSIs) are the most common type of healthcare-associated infections.[10] Despite numerous advancements in asepsis, antibiotics, sterilization, and surgical techniques, SSIs continue to pose a significant challenge across all surgical specialties in hospitals.[11] Wound dehiscence is the separation of skin edges and may vary from partial to complete separation.[12] The Centers for Disease Control and Prevention (CDC) states that wound dehiscence can result in superficial or deep tissue injury and can be linked to SSIs.[3] Furthermore, multidrug-resistant organisms (MDROs) have become a major challenge in the healthcare sector, posing a significant threat to patient outcomes.[13] MDROs that are well-recognized and cause significant global issues include Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant Enterococci (VRE), penicillin-resistant pneumococci, Extended-spectrum beta-lactamase (ESBL) producing *Klebsiella pneumoniae*, and carbapenem-resistant *Acinetobacter baumannii* (CRAB). The emergence of MDROs is influenced by several factors, with the most significant being the use of antibiotics and infection control practices. Therefore, the rational use of antibiotics is crucial.[14]

This retrospective study aimed to elucidate the distribution of microbial diversity and the patterns of antimicrobial susceptibility of bacteria isolated from specimens of patients with wound dehiscence at Prof. Dr. I.G.N.G. Ngoerah General Hospital from 12 January 2020 to 12 December 2022. Even though numerous studies have already described bacteria and their antimicrobial susceptibility patterns, regular and location-specific reports are crucial.

The most commonly identified bacteria in this study were *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. This finding aligned with a study conducted in Turkey by Isik et al.,[9] which showed that *Escherichia coli* was the most frequently detected microorganism in wound cultures (22.8%). other studies conducted in Indonesia have also reported *Escherichia coli* as the predominant microorganism in surgical wound infections (20%).[15] However, another study showed that *Staphylococcus aureus* (28.6%) was the leading causative microorganism of surgical site infection, followed by *Escherichia coli* (24.7%) and *Pseudomonas* spp. (23.7%). These differences could be attributed to variations in sample selections.[16]

The bacteria isolated in this study showed the highest susceptibility to amikacin (72.1%), followed by meropenem (71.4%) and piperacillin/tazobactam (65.7%). The Gram-positive bacteria in this study were found to be most sensitive to linezolid (96.6%), tigecycline (96.6%), and vancomycin (96.6%), aligning with findings from other studies like those by Singh et al.[17] The gram-negative bacteria in this study exhibited the highest sensitivity to amikacin (91%) and meropenem (90.1%), which is comparable to a study in Nepal, where gram-negative bacteria were most sensitive to amikacin (81.8%).[16] Another study in Nigeria reported similar findings, with most gram-negative isolates being susceptible to amikacin (72.7%) and imipenem (72.7%).[17]

This study had the limitation that the sample only included wound dehiscence and SSI cases from inpatients, potentially leading to an underestimation of the overall cases of wound dehiscence and SSIs.

In conclusion, the study found that *Escherichia coli* was the most common bacteria isolated from wound dehiscence cases, and most of the isolates showed susceptibility to amikacin and meropenem. These antibiogram data could provide a basis for selecting empirical therapy for wound dehiscence and SSI cases in local hospital settings, pending definitive culture results.

References

1. Modi J, Patel Y, Trivedi M, Bochiya G. An abdominal wound dehiscence of emergency explorative laparotomy and

their management at tertiary care centre: an observational study. International Surgery Journal. 2023;10(9):1448–54.
2. WUWHS. Surgical Wound Dehiscence: Improving Prevention and Outcomes. World Union of Wound Healing Societies (WUWHS) Consensus Document. London: Wound International; 2018.
3. Sandy-Hodgetts K, Carville K, Leslie GD. Determining risk factors for surgical wound dehiscence: a literature review. Int Wound J. 2015;12(3):265–75. doi:10.1111/iwj.12088
4. WHO. Report on the Burden of Endemic Health Care-Associated Infection Worldwide. Clean Care is Safer Care. Geneva: WHO Library Cataloguing-in-Publication Data; 2011.
5. Pinchera B, Buonomo AR, Schiano Moriello N, Scotto R, Villari R, Gentile I. Update on the management of surgical site infections. Antibiotics. 2022;11(11):1608. doi:10.3390/antibiotics11111608
6. CDC. Procedure Associated Module: Surgical Site Infection (SSI) Event. National Healthcare Safety Network. Atlanta: Centers for Disease Control and Prevention; 2023.
7. Alamrew K, Tadesse TA, Abiye AA, Shibeshi W. Surgical antimicrobial prophylaxis and incidence of surgical site infections at Ethiopian Tertiary-Care Teaching Hospital. Infect Dis (Auckl). 2019;12(1):1–7.
8. Sartelli M, Coccolini F, Carrieri A, Labricciosa FM, Cicuttin E, Catena F. The "torment" of surgical antibiotic prophylaxis among surgeons. Antibiotics. 2021;10(11):1357. doi:10.3390/antibiotics10111357
9. Isik O, Kaya E, Dundar HZ, Sarkut P. Surgical site infection: re-assessment of the risk factors. Chirurgia (Bucur). 2015;110(5):457–61
10. Deka S, Kalita D, Mahanta P, Baruah D, Mahanta Sr P. High prevalence of antibiotic-resistant gram-negative bacteria causing surgical site infection in a Tertiary Care Hospital of Northeast India. Cureus. 2020;12(12):1–10. doi:10.7759/cureus.12208
11. Narula H, Chikara G, Gupta P. A prospective study on bacteriological profile and antibiogram of postoperative wound infections in a tertiary care hospital in Western Rajasthan. J Family Med Prim Care. 2020;9(4):1927–34. doi:10.4103/jfmpc.jfmpc_1154_19
12. Seyffert J, Harding T, Sanghvi A, Bibliowicz N, Yungmann M, Camner S, Leavitt M, Solomon JA. Surgical wound dehiscence following cutaneous excisions: A retrospective study and review of the literature. Journal of Dermatology and Dermatologic Surgery. 2020;24(2):93–8.
13. Njoku CO, Njoku AN. Microbiological pattern of surgical site infection following caesarean section at The University of Calabar Teaching Hospital. Open Access Maced J Med Sci. 2019;7(9):1430–35. doi:10.3889/oamjms.2019.286
14. Ntambi S, Sutiningsih D, Hussein MA, Laksono B. Distribution and prevalence of multidrug-resistant organisms (MDROs) among MDRO-Positive individuals at Dr. Kariadi Hospital. J Epidemiol Kesehat Komunitas. 2023;8(2):103–9.
15. Liana P, Patricia V, Agatha C. Multidrug-resistant organisms (MDRO) patterns of GICU patients in Dr. Mohammad Hoesin Hospital Palembang. J Phys Conf Ser. 2019;1246(1):2.
16. Saffanah NI, Agustina D, Sutejo IR. Postoperative orthopedic surgical site infection antibiogram of dr. Soebandi Hospital Jember in 2019. J Pharm Sci Clin Res. 2020;5(2):110–20.
17. Islam MH, Mishu MP, Afroze N, Mahmud MH, Islam MS, Shah MS. Bacteriological study on surgical site infection in Rangpur Medical College and Hospital. J Rang Med Col. 2022;7(2):12–9.
18. Singh V, Khyriem AB, Lyngdoh WV, Lyngdoh CJ. Surgical site infections - a hospital havoc: retrospective study of surgical site infections in Tertiary Health Care Centre in North East India. IJIRMS. 2018;3(1):1639–43.

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Correlation between Kupffer Cell Infiltration and Liver Parenchymal Cell Damages in Immunosuppressed Drugs-Induced Rats

Agni Febrina Pargaputri, Endah Wahjuningsih, Dwi Andriani
Department of Oral Biology, Universitas Hang Tuah, Surabaya, Indonesia

Abstract

The liver is the largest organ in the body, composed of both parenchymal and non-parenchymal cells. Chemical substances and various drugs can induce liver injury and involve Kupffer cells which are non-parenchymal cells that release biologically active substances, promoting pathological processes. This study aimed to evaluate the correlation between the number of Kupffer cells and liver parenchymal cell damages in immunosuppressed, drug-induced rats. The study was conducted from July to December 2019 at the Oral Biology Laboratory of the Faculty of Dentistry and the Biochemical Laboratory of the Faculty of Medicine at Universitas Hang Tuah Surabaya. Twelve healthy male Wistar rats were divided into two groups: Healthy (H) and Immunosuppressed Drug-Induced (ID) groups. Immunosuppression was induced using dexamethasone (0.5 mg/day/rat), administered orally for 14 days, combined with tetracycline (1%/day/rat). Liver samples from all rats were examined for Kupffer cell count and parenchymal cell damages were assessed using a light microscope with 400x magnification. Results revealed a significant difference in the number of Kupffer cells and liver parenchymal cell damages between the H and ID groups ($p<0.05$). Pearson correlation analysis indicated a significant correlation between Kupffer cell number and parenchymal cell damages ($p=0.000$). Continuous administration of immunosuppressive drugs may activate Kupffer cells, leading to damage of liver parenchymal cells. In conclusion, the infiltration of Kupffer cells is associated with liver parenchymal cell damages, mediated by various factors in the immunosuppressed drug-induced rat model.

Keywords: Immunosuppressed drugs, Kupffer cell, liver parenchymal cell

Introduction

The liver is the largest organ in the body which performs critical and interrelated functions.[1] Liver was made up mostly of parenchymal cells, called hepatocytes, which composed about 78 % of the liver volume, and approximately 6,3 % of non-parenchymal cells.[2] Major functions of the liver include blood filtration and retention; metabolism of carbohydrates, fats, proteins, hormones, and foreign substances; bile formation; storage of vitamins and iron; and the synthesis of coagulation factors.[1,3] Liver also arranges the flux and safety of the compounds that enter the circulation system.[2]

Immunosuppressant drugs are commonly prescribed for various chronic conditions such as rheumatological, neurological, and dermatological conditions, and also reduce the chances of the body rejecting the transplanted organ.[4,5] Immunosuppressants are also known to be the most prominent contributing factor causing harmful responses. Hepatotoxicity actuated by immunosuppressants is troublesome to assess since these drugs are in some cases utilized to treat liver illnesses.[6] The use of long-term and excessive immunosuppressant drugs is known to damage the liver parenchymal cells which will then degrade liver function.[3,9] Previous studies showed that the immunosuppressant drugs administration in 14 days given orally can influence liver albumin levels and serum aminotransferase enzyme levels in oral candidiasis rats model.[10,11] It indicated an impaired liver function and influenced hepatocyte function which crucial to perform drug metabolism.[1,11]

Kupffer cells also called reticuloendothelial cells, tissue macrophages which are distributed along the liver sinusoid, are one of the non-parenchymal cells that have a crucial role in hepatic and systemic homeostasis.[1,2,7] These cells

Corresponding Author:
Agni Febrina Pargaputri
Department of Oral Biology, Universitas Hang Tuah, Jl. Arief Rahman Hakim No. 150, Surabaya, Indonesia
Email: agni.febrina@hangtuah.ac.id

are essential in the pathogenesis of liver disease. They have a role in modulating inflammation, arranging the process of tissue repair, angiogenesis, and fibrosis.[7] Immunosuppressant drugs act as chemical substances that may induce cell damage and injury in the liver.[11] These drugs are digested and absorbed, which then enters the liver with the bloodstream.[12] Chemical substances and several drugs are known to involve Kupffer cells to release biologically active substances that promote pathological processes and induce liver injury.[6] Active Kupffer cells are the main source of inflammatory mediators in injured and necrosis hepatocellular, which can exhibit an increase in cytotoxicity and chemotaxis.[7] The release of inflammatory mediators, growth factors, and reactive oxygen species as the results of toxic agents can activate Kupffer cells, which subsequently have a navel role in liver response to toxicity.[13] Reactive oxygen species (ROS) produced by Kupffer cells are also linked to the induction of inflammation in the liver.[14,15] Persistent inflammation can lead to a reduction in hepatocytes and subsequent tissue damage. Kupffer cells, which function as key custodians in the liver, play a significant role in the initiation and regulation of cirrhosis and liver fibrosis. They contribute to chronic liver inflammation by inducing hepatic stellate cell myofibroblastic transformation, which is associated with the production of ROS, cytokines, and growth factors. The production and regulation of metalloproteinases by Kupffer cells further disrupt homeostatic mechanisms in the deposition of the extracellular matrix, contributing to liver fibrosis.[15]

Several in vitro studies have been developed to assess hepatotoxicity in drug-induced liver injury using primary human hepatocytes and hepatocyte-like cells derived from induced pluripotent stem cells.[16] Research involving experimental animal models and initial clinical trials in humans suggests that targeting Kupffer cells could represent a promising therapeutic approach for treating both acute and chronic liver diseases.[11] Understanding the harmful effects of Kupffer cells in drug-induced liver injury may encourage the expansion of therapeutic strategies to enhance clinical outcomes in liver injury. To date, there has been no research specifically addressing the relationship between the number of Kupffer cells and liver parenchymal cell damage induced by immunosuppressive drugs. Therefore, this study aims to investigate the correlation between Kupffer cell infiltration and liver parenchymal cell damage in immunosuppressive drug-induced rat models.

Methods

The present study used a true experimental design with a post-test-only control group. Research was conducted from July to December 2019 at the Oral Biology Laboratory of the Faculty of Dentistry and the Biochemical Laboratory of the Faculty of Medicine at Universitas Hang Tuah Surabaya. The subjects were healthy male Wistar rats (*Rattus norvegicus*), aged 6 months and weighing between 180 and 200 grams. Sample size calculations were performed using the Higgins formula (1991), which determined that 6 rats per group were needed. Sampling was done using simple random sampling. All rats were acclimated for seven days before the study commenced and were then randomly assigned to two groups: the Healthy (H) group and the Immunosuppressed Drug-Induced (ID) group.
The induction of immunosuppressed drugs was conducted on day 8 using dexamethasone 0.5 mg/day/rat orally for 14 days, added with tetracycline 1 %/day/rat to prevent bacterial infection.[17,18] The dose of dexamethasone was reduced to 0.05mg/day/rat and 0.1%/day/rat for tetracycline on day 11.[17]

At the end of the study, all rats were euthanized. The rats were administered ketamine (10 mg/kg body weight) and diazepam (5 mg/kg body weight) for euthanasia, and their livers were subsequently extracted. Histopathological preparations were prepared using Hematoxylin and Eosin (HE) staining.[19,3] The number of Kupffer cells and liver parenchymal cell damage were observed using a 400x magnification light microscope with 5 fields of view.[3] Kupffer cells are depicted as amoeboid-shaped cells with oval nuclei and elongated cytoplasmic processes, attached or close to the sinusoid.[20] The examination of liver parenchymal cell damage was carried out by counting cells that show features of karyolysis, karyorrhexis, and pycnotic.[3]

The normality of the data was assessed using the Shapiro-Wilk test, and homogeneity was evaluated with the Levene test. The difference in the number of Kupffer cells and liver parenchymal cell damage between the two groups was analyzed using an independent sample t-test, with significance set at $p<0.05$. The correlation between Kupffer cell count and liver parenchymal cell damage was determined using

Pearson's correlation test, with significance set at $p<0.01$.

This research was approved by the Research Ethics Committee of the Faculty of Dentistry, Universitas Hang Tuah Surabaya (No: EC/010/KEPK-FKGUHT/VII/2019).

Results

The Shapiro Wilk test showed the data were normally distributed and the Levene statistical test showed homogeneous data ($p>0,05$). The number of Kupffer cells in ID group increased compared to H group, as well as the number of liver parenchymal cell damage (Table 1). The number of Kupffer cells and liver parenchymal cell damage was significantly different in H group when compared with ID group (Table 1).

The analysis of the relationship between the number of Kupffer cells and liver parenchymal cell damage was conducted using a Pearson correlation test, which revealed a strong positive correlation with a correlation coefficient (r) of 0.943. This result was statistically significant, with a p-value of 0.000 ($p<0.01$).

Discussion

This study revealed an increase in the number of Kupffer cells and liver parenchymal cell damage following the administration of immunosuppressive drugs. These findings suggest that prolonged use of immunosuppressive drugs may lead to the activation of Kupffer cells, resulting in subsequent damage to liver parenchymal cells. In contrast, previous research indicated a reduction in Kupffer cell numbers following acetaminophen induction.[21] In acetaminophen-liver injury, Kupffer cells play protective and pro-repairing roles by recovering hepatocyte proliferation and preventing necrosis.[22] However, several prior studies revealed the elevation of activation Kupffer cells due to an increase of endotoxin and administration of carbon tetrachloride (CCl4) in animal models.[23] The microenvironment shapes the unique immunosuppressive features and functions of Kupffer cells.[23] An early hepatocellular injury can occur in reaction to the enactment of adaptive and innate immune responses in drug-induced liver injury.[24] The complexity of the immune system can arise because the liver is an unique immunological organ that houses multiple innate and adaptive immunity which are complementary and interrelated.[23] The reaction of the innate and adaptive immune system could be a key component leading to liver damage caused by chemical agents.[6] The maintenance of the equilibrium between host defense against pathogenic agents and harmless antigen resistance becomes a crucial role of innate immune cells within the liver including Kupffer cells. In any case, when exposure to certain agents, or infections is present, the equilibrium can be upset, leading to the activation of Kupffer cells.[24]

Corticosteroids as immunosuppressed drugs used in this study may act as chemical substances that induce the activation of Kupffer cells. The excessive amount of drugs that enter the liver, will activate the Kupffer cells to execute its function in getting rid of foreign chemicals and particles from portal circulation system into the liver efficiently.[20] Thereby, Kupffer cells reside in liver sinusoids also have a role in phagocytic function, enabling them to get rid of bacteria, viruses, and cellular debris derived from the arterial and portal circulation.[25,26] Kupffer cells also act as early defense against particle and immunoreactive substances that enter the gastrointestinal tract through portal circulation.[26] Besides phagocytic function, Kupffer cells are able to release cytokines inflammatory, oxygen radicals, and proteases, which may be involved in the development of liver injury.[20]

The correlation analysis in this study revealed a significant and strong association between the number of Kupffer cells and liver parenchymal cell damage. This finding suggests

Table 1 The Kupffer Cells and Liver Parenchymal Cell Damage Number in Each Group

Variable	H Group	ID Group
Kupffer cells	3.8 ± 1.1^{a}	7.96 ± 6.2^{b}
Liver parenchymal cell damage	16.2 ± 1.9^{a}	146.6 ± 25.9^{b}

*a,b: The different superscript letters on the same line showed significant differences at level $p<0.05$

that the administration of immunosuppressive drugs may induce Kupffer cell activation, which in turn can contribute to liver parenchymal cell damage. The positive correlation indicates that an increase in Kupffer cell numbers is associated with a corresponding increase in parenchymal cell damage. The study implies that prolonged use of immunosuppressive drugs may lead to Kupffer cell activation, which can trigger the expression of pro-inflammatory cytokines and chemokines. If these inflammatory mediators are not adequately regulated, they may ultimately result in liver cell damage.[7,27] Cytokines such as interleukin-6 (IL-6), tumor necrosis factor α (TNFα), and chemokines are released by Kupffer cells, leading to acute inflammation.[24] Mitochondrial DNA from apoptotic parenchymal cells activate the STING/NF-kB signaling pathway in Kupffer cells and leads to amplification of inflammation. Several inflammation cells such as monocytes and neutrophils are recruited by Kupffer cells through CCL2 and CXCL1 which can worsen the inflammation.[23] Activated Kupffer cells in this study resulted in the release of proteolytic enzymes which are known to cause liver damage in mouse models.[28] The release of reactive oxygen species and proteases by Kupffer cells can induce necrotic cell damage by extravasating and adherence of neutrophils to parenchymal cells after chemotactic stimulation.[7] Reactive oxygen species induce inflammation through the enhancement of transcription factor NF-kB activation, which regulates the formation of cytokines and chemokines, which subsequently cause cell injury and contribute to the liver damage severity.[7,29]

A limitation of this study is the lack of comparison regarding the duration of immunosuppressive drug administration. Further research is needed to assess how varying lengths of drug exposure impact liver health, both positively and negatively. In conclusion, Kupffer cell infiltration is associated with liver parenchymal cell damage in drug-induced immunosuppression in rat models. Kupffer cells activate various mediators, cytokines, and reactive oxygen species, which can induce damage to liver parenchymal cells in these models.

References

1. Guyton and Hall. Textbook of medical physiology. 14th ed. Singapore: Elsevier; 2021. p. 871.
2. Mohammed K. Hassani. Liver structure, function and its interrelationships with other organs: a review. International Journal Dental and Medical Sciences Research. 2022;4(1):88–92. doi: 10.35629/5252-04018892
3. Pargaputri AF, Andriani D, Hartono MR, Widowati K. Effect of hyperbaric oxygen treatment on liver hepatocyte damage in oral candidiasis immunosuppressed rats. Odonto: Dental Journal. 2022;9(2):319–26. doi: 10.30659/odj.9.2.319-326
4. Yaseen H, Haroon K. Immunosuppressive drugs. Encyclopedia of Infection and Immunity. 2022:726–40. doi: 10.1016/B978-0-12-818731-9.00068-9
5. Fenella BP, Thura WH, Mina K, Phyo KM. Systematic review of immunosuppressant guidelines in the COVID-19 pandemic. Ther Adv Drug Saf. 2021;12:2042098620985687. doi: 10.1177/2042098620985687
6. Elena T, Judit C, Mercedes R, MA Isabel Lucena, Raúl JA. Hepatotoxicity induced by new immunosuppressants. Gastroenterol Hepatol. 2010;33(1):54–65. doi: 10.1016/j.gastrohep.2009.07.003.
7. Hiroko T, Shuhei N. Importance of kupffer cells in the development of acute liver injuries in mice. Int. J. Mol. Sci. 2014;15:7711–30. doi:10.3390/ijms15057711
8. Prasetiawan E, Sabri E, Ilyas S. Gambaran histologis hepar mencit (mus musculus l.) strain ddw setelah pemberian ekstrak n-heksan buah andaliman (Zanthoxylum acanthopodium Dc.) selama masa pra implantasi dan pasca implantasi. Saintia Biol. 2012;1(1):40–5.
9. Pargaputri AF, Andriani D. Serum albumin levels of oral candidiasis immunosuppressed rats treated with hyperbaric oxygen. International Journal of Integrated Health Sciences. 2020;8(1):8–13. doi: 10.15850/ijihs.v8n1.2086
10. Pargaputri AF, Andriani D. Hepatocellular liver function of immunosuppressed rats with oral candidiasis after hyperbaric oxygen treatment: Alanine transaminase and aspartate transaminase levels. Arch Orofac Sci. 2021;16(Supp.1):5–9. doi: 10.21315/aos2021.16.s1.2
11. Oliver K, Frank T. Liver macrophages in tissue homeostasis and disease. Nature Reviews Immunology. 2017;17:306–21. doi: 10.1038/nri.2017.11
12. Meurman JH, Siikala E, Richardson M, Rautemaa R. Non-Candida albicans Candida

yeasts of the oral cavity. In: Méndez-Vilas A (ed.), Communicating Current Research and Educational Topics and Trends in Applied Microbiology. 2017:719–31.
13. Ruth AR, Patricia EG, Cynthia J, Lisa MK, Ivan R, James EK. Role of the Kupffer cell in mediating hepatic toxicity and carcinogenesis. Toxicol Sci. 2007;96(1): 2–15. doi: 10.1093/toxsci/kfl173.
14. Elise Slevin, Leonardo Baiocchi, Nan Wu, Burcin Ekser, Keisaku Sato, Emily Lin, et al. Kupffer cells: inflammation pathways and cell-cell interactions in alcohol-associated liver disease. Am J Pathol. 2020;190(11):2185–93. doi: 10.1016/j.ajpath.2020.08.014.
15. Subhra KB and Alberto M. Kupffer Cells in Health and Disease. Macrophages: biology and role in the pathology of diseases. 2014:217–47.
16. M. Teresa Donato, Gloria Gallego-Ferrer, and Laia Tolosa. In vitro models for studying chronic drug-induced liver injury. Int. J. Mol. Sci. 2022;23(19):11428. doi: 10.3390/ijms231911428
17. Pargaputri AF, Andriani D. The effect of hyperbaric oxygen therapy to the amount of lymphocytes in oral candidiasis immunosuppressed model. Denta J Kedokt Gigi. 2018;12(2):36–44.
18. Andriani D, Pargaputri AF. Enhance of il-22 expression in oral candidasis immunosuppressed model with acanthus ilicifolius extract therapy. IOP Conf Ser Earth Environ Sci. 2019;217:012056.
19. Paramita DO, Endah W, Dwi A. Efektivitas suplementasi teripang emas (Stichopus hermanii) dalam mencegah terjadinya oral candidiasis pada tikus wistar. Denta 2018; 12(1):9–15. doi: 10.30649/denta.v12i1.155
20. Hajira Basit, Michael L. Tan, Daniel R. Webster. Histology, kupffer cell. Treasure Island (FL): StatPearls Publishing; 2022.
21. David BA, Rezende RM, Antunes MM, Santos MM, Freitas LM, Diniz AB, et al. Combination of mass cytometry and imaging analysis revealsorigin, location, and functional repopulation of liver myeloid cells in mice. Gastroenterology. 2016;151:1176–91. doi: 10.1053/j.gastro.2016.08.02415.
22. Kopec AK, Joshi N, Cline-Fedewa H, Wojcicki AV, Ray JL, Sullivan BP, et al. Fibrinogen drives repair after acetaminophen-induced liver injury via leukocyte alphambeta2 integrin-dependent upregulation of mmp12. J Hepatol. 2017;66:787–97. doi: https://doi.org/10.1053/j.gastro.2016.08.024.
23. Li W, Chang N, Li L. Heterogeneity and function of kupffer cells in liver injury. Front. Immunol. 2022;13:940867. doi: 10.3389/fimmu.2022.940867
24. Farah Tasnim, Xiaozhong Huang, Christopher ZWL, Florent Ginhoux, and Hanry Yu. Recent advances in models of immune-mediated drug-induced liver injury. Front. Toxicol. 3: 605392. doi: 10.3389/ftox.2021.605392
25. Takashi Sakai and Wen-Ling Lin. Kupffer cells as a target for immunotherapy. sakai t, lin w-l. kupffer cells as a target for immunotherapy. J Multidisciplinary Scientific Journal. 2022;5:532–7. doi: 10.3390/j5040036
26. Laura JD, Mark B, Hui T, Michele TP, Laura EN. Kupffer cells in the liver. Compr Physiol. 2013;3(2):785–97. doi: 10.1002/cphy.c120026
27. Mark B, Laura JD, Zhang-Xu L, Hui T, Laura EN. Macrophages and kupffer cells in drug-induced liver injury. Drug-Induced Liver Disease (Third Edition). 2013. Academic press. p: 147-155. doi: 10.1016/B978-0-12-387817-5.00009-1
28. Reham Hassan. Kupffer cells in hepatotoxicity. EXCLI Journal. 2020;19:1156-1157.
29. Kendran AAS, Arjana AA, Pradnyantar AASI. Aktivitas enzim alanine-aminotransferase dan aspartate aminotransferase pada tikus putih jantan yang diberi ekstrak buah pinang. Buletin Veteriner Udayana. 2017;9(2):132–8.

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Effect of Saline Nasal Irrigation in Patients with Sinonasal Symptoms

Asti Widuri, Deoni Daniswara, Rizka Fakhriani
Department of Otorhinolaryngology-Head and Neck, Faculty of Medicine and Health Science, Universitas Muhammadiyah Yogyakarta, Indonesia

Abstract

Nasal irrigation with saline is frequently used as an alternative therapy for sinonasal diseases since it effectively reduces sinonasal symptoms and improves the quality of life when measured by various subjective instruments. The mechanisms of action of saline irrigation remain unclear, but some hypotheses attribute the direct physical cleansing of thick mucus, allergens, and pollutants. This study aimed to determine the effect of nasal saline irrigation on the nasal mucosa using the modified Lund-Kennedy endoscopic score as the objective evaluation. This pre-post-experimental study was conducted at PKU Muhammadiyah Gamping Hospital Yogyakarta, Indonesia, from May to June 2023. The study included 18 individuals presenting with sinonasal symptoms. Participants meeting the inclusion and exclusion criteria who provided informed consent were trained in nasal saline irrigation. Patients were assessed at baseline and one week after nasal saline irrigation using endoscopy, TNSS, and MCC time. Results revealed that the mean of endoscopic scores before and after saline irrigation were 6.89±1.1 and 5.33±1.4, respectively (p-value 0.006), while the mean of TNSS scores before and after saline irrigation was 6.17±2.3 and 4.28±2.6, respectively (p-value 0.006). Thus, nasal saline irrigation significantly improves the endoscopy and TNSS scores, but not the MCC time.

Keywords: Allergen, endoscopy, human, nasal mucosa, quality of life

Introduction

The history of nasal irrigation is believed to be from the ancient Hindu practice of Ayurveda, the yogic system of body cleansing techniques in India, during the mid-first millennium BC. The procedure of jala neti involves flushing the nasal cavity by using water poured into one of the nostrils and out into the other. Over the past two decades, Western medicine has adopted nasal irrigation, gaining popularity worldwide.[1] Some extensive study have been conducted to assess the beneficial effect of nasal irrigation in treating sinonasal diseases, the recent development of the basic science behind and the emergence of new technologies.

Nasal irrigation has been an easy procedure, physiotherapy, inexpensive budget, and safe as an adjuvant treatment for sinus and nasal conditions for many years. It is still recommended by physicians for sinonasal diseases such as acute rhinitis, allergic rhinitis, rhinosinusitis, and post-sinus surgery. Nasal irrigation successfully lessens the symptoms and indicators of sinonasal disorders. Additionally, it was recommended to cure and avoid developing upper respiratory infections. Every sinonasal condition should have a different approach to saline irrigation to achieve therapeutic benefits; large, volume devices are more effective for allergic rhinitis, but low-volume devices are for children. Nasal saline irrigation is more beneficial for children with acute rhinosinusitis, although it is also an option for adults.[2]

The mechanism of nasal irrigation on nasal mucosa is divided into physical and biological or physiological activity.[1] The physical impact of clearing the mucosa of excessive secretion minimizes the concentration of particle pollutants and pathogens.[3] The biological effect depends on the ion activities on the mucosal cells.[4] Saline solutions will hydrate and moisten the nasal mucosa, causing mucous to change from gel to sol, requiring less energy for cilia to beat and enhancing mucociliary clearance.[5] Many minerals showed another effect, such as

Corresponding Author:
Rizka Fakhriani
Department of Otorhinolaryngology-Head and Neck, Faculty of Medicine and Health Science, Universitas Muhammadiyah Yogyakarta, Indonesia
Email: rizkafakhriani@umy.ac.id

boosting the viability of cells and minimizing inflammatory conditions.[6]

The measurement of sinonasal medical treatment efficacy using standardized related patient-reported outcomes (symptom scores, medication scores, disease control scores, and satisfaction or quality-of-life scales) is widely used as a subjective assessment.[7] The modified Lund-Kennedy endoscopic score is one of the validated objective outcome measures used to stage the inflammation of sinonasal mucosa.[8] This research combine subjective assessment and objective assessment, provides by assessing the impact of nasal irrigation with saline on nasal mucosal using an endoscopic assessment score.

Methods

A quasi-experimental pretest-posttest design study was conducted at PKU Muhammadiyah Gamping Hospital Yogyakarta, Indonesia, from May to June 2023. The study hypothesized that saline nasal irrigation significantly improved nasal mucosa condition. The assessment performed an endoscopy examination to test the hypothesis, with mucociliary function as the objective outcome and total nasal score symptoms (TNSS) as a subjective outcome.

The participants were recruited using a consecutive sampling method considering the inclusion criteria, such as adult patients, females, and males who complain of sinonasal symptoms and are willing to participate with an informed consent sign. At the same time, the exclusion criteria included those who suffer sinonasal complications, including polys nasi and rhinosinusitis. The sample in this study was calculated using the research sample size formula for paired numerical comparative analytical tests for 2 groups, and added with 10% dropout criteria. The results obtained for the number of samples for preliminary research was 20 participants.

The instrument of the primary outcome assessment using endoscopy examination and endoscopic scoring was utilized using the modified Lund-Kennedy system with three domains; the first domain was discharge (0 = no discharge; 1 = clear thin discharge; 2 = thick purulent discharge), the second domain was swelling (0 = no swelling; 1 = mild swelling; 2 = severe swelling), and the third domain was polyp (0 = no polyp; 1 = polyp only in the middle nasal meatus; 2 = polyp exceeding the middle nasal meatus).[9] The secondary outcome of nasal function was examined using a saccharin test to measure mucociliary clearance time. The subject sat straight, and 2 mm saccharin powder was applied to the anterior end of the inferior turbinate, 1 cm beneath the top of the inferior concha. The subject's first sense of a sweet taste was documented.

The Total Nasal Symptoms Score (TNSS) questionnaire was used to assess the subjective outcome, which comprised four symptom categories: rhinorrhea, nasal obstruction, nasal itching sensation, and sneezing. There is a 4-point scale for each symptom category: 0 is none, 1 is mild, 2 is moderate, and 3 is severe.[10] Adverse events and troubles related to nasal irrigation were also monitored using participant self-report.

Before standardized nasal irrigation was performed, participants were given training first. Participants performed nasal washing using a solution they made by mixing 0.9% NaCl from clear sodium chloride dissolved in 3.1 grams with boiled water into 300 ml in a flowing nasal rinse bottle. Both nasal cavities were irrigated using 150 ml on each side once a day for one week. Before treatment, the participants blew their mucous; then, their heads were positioned forward. The bottle's cannulas were laid on the nostril and slowly flowed into the nasal cavity. The saline solution emerged through the mouth after entering the nasopharynx. The other nasal cavity received similar irrigation.

The participants who completed the study were used to analyze the efficacy assessments. The quantitative results were reported as mean and standard deviation and the qualitative information was percentages. Before analyzing the data, a data normality test was conducted to determine what type of analysis would be used. This study used the Shapiro-Wilk normality test because the number of respondents was less than 30. For comparing quantitative data before and after the test, the student's paired t-test was utilized; a p-value of 0.05 was interpreted as statistically significant.

The Faculty of Medicine and Health Sciences Research Ethics Committee of Universitas Muhammadiyah Yogyakarta granted formal approval for this study with the reference number 115/EC-KEPK FKIK UMY/II/2023.

Results

This study involved 18 participants (2 participants lost of follow-up), which

Table 1 Characteristic of Subjects

	Characteristic Respondents	n=18	%	Total
Gender	Male	8	44.4	18
	Female	10	55.6	
Age	18–25	0	0	18
	26–35	8	44.4	
	36–60	10	55.6	
SFAR	≥7	13	72.2	18
	<7	5	27.8	
SPT	+	13	72.2	18
	-	5	27.8	
Sibling number	1–2	13	72.2	18
	3–4	3	16.7	
	>4	2	11.1	
Resident	Urban	5	27.8	18
	Rural	13	72.2	
Pet	Yes	7	38.9	18
	No	11	61.1	
Smoke	Yes	3	16.7	18
	No	15	83.3	
Exercise	0	4	22.2	18
	1–2	11	61.1	
	3–4	2	11.1	
	>4	1	5.6	

SFAR=score for allergic rhinitis; SPT=skin prick testing

resulted from consecutive recruitment from ENT polyclinic. According to Table 1, these respondents included eight males (44.4%) and ten females (55.6%) aged 26–60. Participants with sinonasal symptoms, according to Score for Allergic Rhinitis (SFAR), 13 (72,2%) had a≥7 total score, and 5 (27.8%) had a <7 total score. From skin-prick test results, 13 (72,2%) were positive, and 5 (27.8%) were negative (Table 1). The outcomes of the pre-and post-nasal irrigation with saline at seven days in terms of TNSS, endoscopic score, and MCC are shown in Table 2.

Discussion

In this study, nasal saline irrigation showed significantly decreased symptoms of allergic rhinitis according to TNSS scores, it's in line with a previous report that in comparison to no saline irrigation, saline irrigation may lessen the

Table 2 The Outcomes of the Pre-and Post-Nasal Irrigation

Type of Examination		Mean	Std. Deviation	Std Error Mean	p[a]
Endoscopic score	Before	6.89	1.132	0.267	0.006
	After	5.33	1.414	0.333	
Mucociliary clearance time	Before	82.48	154.550	36.428	0.65
	After	105.24	115.578	27.242	
TNSS score	Before	6.17	2.383	0.562	0.006
	After	4.28	2.608	0.615	

TNSS=total nasal symptom; [a] =t-test

severity of disease as reported by the patient in both adults and children. Most sinonasal diseases require to be treated with nasal saline, although each condition should have an optimal delivery device and best solution. Most sinonasal diseases could be treated with nasal saline, although each condition should have an optimal delivery device and best solution.[2] Additionally, it offers a safe, simple, and effective therapy option for pregnant women with seasonal allergic rhinitis.[11]

Nasal saline irrigation has been accepted as adjuvant therapy for sinonasal disorders, including Allergic Rhinitis. It's effective for decreasing nasal symptoms and minimizing the over-usage of medication in both adults and children.[12] Commonly, the clinical success of therapy for sinonasal diseases is measured using validated patient-reported outcome measures and clinical, physiologic measures. The primary outcomes related to sneezing, rhinorrhea, nasal itching, and nasal obstruction were evaluated by the subjective assessment, including Total Nasal Symptom Score (TNSS), Sinonasal Outcome Test (SNOT-22), and Visual Analog Scale (VAS).[13] The objective results help assist in diagnosing and treating a nasal obstruction in sinonasal diseases; they use peak nasal inspiratory flow (PNIF), acoustic rhinometry (AR), and rhinomanometry (RM) to measure various aspects of nasal obstructions.[14] Another objective measurement of nasal condition was an endoscopic score that figured the inflammation process on the nasal mucosa.[8] Nasal saline irrigation may improve endoscopy scores attributed to mechanical intervention on the nasal mucosa. Experts suggest that the way nasal saline irrigation works is by cleaning the nasal mucosa directly. The mechanical action of nasal saline irrigation will help remove inflammatory mediators as well as antigens responsible for allergic reactions, modify the ciliary beat frequency in order to enhance mucociliary clearance (MCC), and soften and remove mucus.[15]

The prevalence of allergic rhinitis in this study was similar between females and males, which aligns with epidemiological research indicating that males are more likely to develop allergic rhinitis during childhood. Conversely, females are more likely to develop it during adolescence, with no significant gender distinction in adulthood.[16]

This study also revealed that 72.2% of respondents were from rural areas. According to the hygiene hypothesis, allergic diseases are typically less frequent in rural regions compared to urban areas. However, it is noteworthy that some rural areas also experience high levels of air pollution, which may contribute to the prevalence of allergic rhinitis. Another reason the rural environment is protective against atopic disease is a rural farming type, the protective effect linked with intimate contact with animals, the consumption of unpasteurized milk, and rising endotoxin exposure is now being investigated, and the hypothesized gene-by-environment interactions involving essential innate immune genes give perspectives on probable mechanisms. It is also similar to the epidemiology report from China that the prevalence of confirmed allergic rhinitis is comparable between rural dan urban locations.

In correlation with sibling number, 72.2 % of respondents have 1–2 siblings, so it's consistent with the hygiene hypothesis theory that smaller families may provide a more sanitary environment and less exposure to microorganisms for young children. Minimal exposure to the environment in childhood could be a risk factor for developing immune hypersensitivities later in life.[17] The Hygiene Hypothesis at that time was consistent with the emerging general theory that non-inherited/ non-communicable diseases like allergies and asthma develop as a result of an improper interaction between environmental exposures and a particular genotype that results in the formation of a particular (disease) phenotype.[18]

Objective endoscopy assessment showed significant improvement in the nasal mucosa of allergic rhinitis according to modified Lund-Kennedy scores (p=0.006). Previous studies showed that using Lund-Kennedy endoscopy scores, high-volume budesonide irrigation is better and safer than normal saline for the post-sinus operation of rhinosinusitis disease.[19]

The respiratory epithelium produces mucus, which collects particles, retaining moisture and ciliated cells (moving numerous cilia to transport mucus into the pharynx). Some studies suggest nasal irrigation directly affects secretions mobilization and increases mucociliary clearance rates.[4,20] Mucociliary Clearance (MCC) time refers to the duration required for cilia to transport particles and mucus out of the nasal cavity. Previous research indicates that the average MCC time ranges from a minimum of 8 minutes to a maximum of 19.2 minutes. Several factors can influence MCC time, including posture, age, sleeping habits, and gender.[21] However, this study found that MCC scores remained similar before and after nasal saline irrigation. The study has several limitations,

including the small sample size and the pre-post-test design. These limitations suggest the need for a larger randomized controlled trial to validate the findings. Based on the research results, the study concluded that there is a significant improvement in the mean endoscopic score following the use of nasal saline irrigation.

References

1. Štanfel D, Kalogjera L, Ryazantsev SV, Hlača K, Radtsig EY, Teimuraz R, et al. The role of seawater and saline solutions in treatment of upper respiratory conditions. Mar Drugs. 2022;20(5):330. doi: 10.3390/md20050330
2. Chitsuthipakorn W, Kanjanawasee D, Hoang MP, Seresirikachorn K, Snidvongs K. Optimal device and regimen of nasal saline treatment for sinonasal diseases: systematic review. OTO Open. 2022;6(2):2473974X221105277. doi: 10.1177/2473974X221105277.
3. Edwards D, Hickey A, Batycky R, Griel L, Lipp M, Dehaan W, et al. A new natural defense against airborne pathogens. QRB Discov. 2020;1:e5. doi: 10.1017/qrd.2020.9.
4. Jiao J, Zhang L. Influence of intranasal drugs on human nasal mucociliary clearance and ciliary beat frequency. Allergy Asthma Immunol Res. 2019;11(3):306–19. doi: 10.4168/aair.2019.11.3.306.
5. Bustamante-Marin XM, Ostrowski LE. Cilia and mucociliary clearance. Cold Spring Harb Perspect Biol. 2017;9(4):a028241. doi: 10.1101/cshperspect.a028241.
6. Grasso M, de Vincentiis M, Agolli G, Cilurzo F, Grasso R. The effectiveness of long-term course of sterimar mn nasal spray for treatment of the recurrence rates of acute allergic rhinitis in patients with chronic allergic rhinitis. Drug Des Devel Ther. 2018;12:705–9. doi: 10.2147/DDDT.S145173.
7. Calderón MA, Casale TB, Demoly P. Validation of patient-reported outcomes for clinical trials in allergic rhinitis: a systematic review. J Allergy Clin Immunol Pract. 2019;7(5):1450–61.e6. doi: 10.1016/j.jaip.2019.01.015.
8. Wangworawut Y, Hwang PH. Modified lund-kennedy score as a predictor for significant symptom improvement after endoscopic sinus surgery short title: MLK Score as a predictor for SSI after ESS. AMJAM. 2021;21(2):106-13.
9. Psaltis AJ, Li G, Vaezeafshar R, Cho K, Hwang PH. Modification of the lund-kennedy endoscopic scoring system improves its reliability and correlation with patient-reported outcome measures. The Laryngoscope. 2014;124(10):2216–23. doi: 10.1002/lary.24654
10. Restimulia L, Pawarti DR, Ekorini HM. The Relationship between serum vitamin D levels with allergic rhinitis incidence and total nasal symptom score in allergic rhinitis patients. Open Access Maced J Med Sci. 2018;6(8):1405–9. doi: 10.3889/oamjms.2018.247
11. Abdullah B, Periasamy C, Ismail R. Nasal irrigation as treatment in sinonasal symptoms relief: a review of its efficacy and clinical applications. Indian J Otolaryngol Head Neck Surg. 2019;71(Suppl 3):1718–26. doi: 10.1007/s12070-017-1070-0
12. Wang Y, Jin L, Liu SX, Fan K, Qin ML, Yu SQ. Role of nasal saline irrigation in the treatment of allergic rhinitis in children and adults: A systematic analysis. Allergol Immunopathol (Madr). 2020;48(4):360–7. doi: 10.1016/j.aller.2020.01.002
13. Zhang K, Li AR, Miglani A, Nguyen SA, Schlosser RJ. Effect of medical therapy in allergic rhinitis: a systematic review and meta-analysis. Am J Rhinol Allergy. 2022;36(2):269–80. doi: 10.1177/19458924211041438.
14. de Oliveira GMM, Correia Júnior MAV, Costa EC, Lira GVAG, Rizzo JÂ, Hunter S, et al. Accuracy of peak nasal flow to determine nasal obstruction in patients with allergic rhinitis. Acta Otorhinolaryngol Ital. 2022;42(2):155–61. doi: 10.14639/0392-100X-N1617.
15. Park DY, Choi JH, Kim DK, Jung YG, Mun SJ, Min HJ, et al. Clinical practice guideline: Nasal irrigation for chronic rhinosinusitis in adults. Clin Exp Otorhinolaryngol. 2022;15(1):5–23. doi: 10.21053/ceo.2021.00654
16. Rosario CS, Murrieta-Aguttes M, Rosario NA. Allergic rhinits: impact on quality of life of adolescents. Eur Ann Allergy Clin Immunol. 2021;53(6):247–51. doi: 10.23822/EurAnnACI.1764-1489.176
17. Pierau M, Arra A, Brunner-Weinzierl MC. Preventing atopic diseases during childhood - early exposure matters. Front Immunol. 2021;12:617731. doi: 10.3389/fimmu.2021.617731
18. Han X, Krempski JW, Nadeau K. Advances and novel developments in mechanisms of allergic inflammation. Allergy. 2020;75(12):3100–11. doi: 10.1111/all.14632

19. Tait S, Kallogjeri D, Suko J, Kukuljan S, Schneider J, Piccirillo JF. Effect of budesonide added to large-volume, low-pressure saline sinus irrigation for chronic rhinosinusitis: a randomized clinical trial. JAMA Otolaryngol Head Neck Surg. 2018;144(7):605–12. doi: 10.1001/jamaoto.2018.0667
20. Sauvalle M, Alvo A. Effect of the temperature of nasal lavages on mucociliary clearance: a randomised controlled trial. Eur Arch Otorhinolaryngol. 2018;275(9):2403–6. doi: 10.1007/s00405-018-5060-y
21. Passali D, Cappello C, Passali GC, Cingi C, Sarafoleanu C, Bellussi LM. Nasal muco-ciliary transport time alteration: efficacy of 18 B Glycyrrhetinic acid. Multidiscip Respir Med. 2017;12(1):29. doi: 10.1186/s40248-017-0110-7

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Nephroprotective Effects of Sunkist Peel Ethanol Extract on Diabetic Nephropathy

Maya Sari Mutia, Chrismis Novalinda Ginting, OK Yulizal, Suhartomi, Widya Yanti Sihotang
Faculty of Medicine, Dentistry and Health Science, Universitas Prima Indonesia, Medan, Indonesia

Abstract

The most common long-term diabetic complication is diabetic nephropathy which, in most cases, falls into the End-Stage Kidney Disease. Numerous studies on the nephroprotective effects of citrus fruit have been conducted recently. This study was performed to investigate the phytochemical properties of Sunkist peel ethanol extract and their possible effects on improving diabetic nephropathy. To get the extract, the Sunkist peels were macerated and the extract was analyzed by GC-MS. An in vivo assay of five different groups of 25 male Wistar rats was then performed using the extracts. The groups included control, standard, 500 mg/kg extract, 750 mg/kg extract, and 1,500 mg/kgBW extract. All rats were sacrificed after 28 days of treatment and VEGF level serum and kidney histology analysis were performed. The VEGF data were first analyzed descriptively, followed by one-way ANOVA or Kruskal-Wallis test based on the data distribution. Meanwhile, the kidney histology was observed and narratively described. Results showed that the Sunkist peel ethanol extract has a potential effect to suppress the VEGF level (p-value<0.05). The extract at the highest dose revealed the mildest pathology damage in the form of tubular structure degeneration without any glomerular damage. This study indicates that Sunkist peel extract has nephroprotective effects from its various phytochemical compounds that work as antioxidants and anti-inflammation.

Keywords: Diabetic, nephropathy, Sunkist peel, vascular endothelial growth factor

Introduction

Indonesia was reported as the 7th highest diabetic patient in 2019 by the International Diabetes Federation (IDF) after China, India, the United States, Brazil, Russia, and Mexico. Moreover, Saeedi et al. also estimated the prevalence of diabetes for 2030 and 2045 from IDF Atlas 9th edition. The estimated global population with diabetes mellitus is 9.3% aged 20–79 years in 2019, and it was predicted to rise to 10.2% by 2030 and 10.9% by 2045. Hence, it is evident that diabetic Mellitus is a chronic disease in some countries all over the world.[1,2]

Diabetes mellitus is a chronic metabolic disease that involves insulin dysfunction and impacts the blood glucose level. Prolonged high blood glucose levels may cause either microvascular or macrovascular complications. One of these microvascular is nephropathy.[3] The most common long-term diabetic complication was diabetic nephropathy, and most of these cases fall into End-Stage Kidney Disease (ESKD).[4] Ironically, around 50% of ESKD was found in some developing countries. Due to this reason, diabetic nephropathy is an essential health problem, so it has become important to develop various treatment modalities to prevent the complications of diabetes, especially diabetic nephropathy.[5]

Some studies have been performed to investigate various herbs for diabetic treatment. However, a limited number of studies looked for the nephroprotective effect of herbs against diabetic nephropathy. One of these herb that have been investigated for antidiabetic was Sunkist peels. Sunkist from the *Citrus* family reported various phytochemical contents like ascorbic acid, flavonoid, phenolic compounds, and pectin. Moreover, Suhartomi et al reported that *Citrus sinensis,* either as the crude extract or fraction, had numerous flavonoid contents that had either DPPH or Hydrogen peroxide scavenging activity as antioxidant properties.[6] Total Flavonoids in *Citrus sinensis* Ethanol and Ethyl acetate extract was 205.66±49.63 mg QE/gr. DW and 242.48±13.83 mg QE/gr. DW,

Corresponding Author:
Maya Sari Mutia
Faculty of Medicine, Dentistry and Health Science, Universitas Prima Indonesia, Medan, Indonesia
Email: mayasarimutia@unprimdn.ac.id

respectively. Depari et al reported that the Sunkist peel extract have antidiabetic activity and improve the hypercholesterolemia state in streptozotocin-induced diabetic rats. [7] Moreover, Mutia et al reported an advanced effect of Sunkist ethanol peels to extract other than antidiabetic which is the nephroprotective effect of the Sunkist peels ethanol extract against diabetic nephropathy by decreasing Blood Urea Nitrogen (BUN) and creatinine levels of Streptozotocin-Induced diabetic rats. [8,9]

Based on these previous studies, none of these studies looked for the nephroprotective effect and exact mechanism of action from ethanol extract as a nephroprotective effect against the nephropathy diabetic. It is hypothesized that the peel of Sunkist suppresses inflammation and oxidative stress associated with diabetic neuropathy through its antidiabetic, anti-inflammatory, and antioxidant effects. This study was conducted to analyze the phytochemical properties of an ethanol extract of Sunkist peels and to assess its potential for improving diabetic nephropathy in male Wistar rats through an animal trial.

Methods

The research utilized an experimental study with a post-test control group design, conducted in the Pharmacology Laboratory of Universitas Prima Indonesia in June 2021. The study procedures were approved by the Health Research Ethics Committee under letter no. 020/KEPK/UNPRI/II/2021. Materials used in the study included Sunkist peel, ethanol, sodium carboxymethyl cellulose, distilled water, streptozotocin, rat pellets, phytochemical screening reagents, metformin tablets, chloroform, 10% buffered formalin solution (BFS), normal saline, xylol, concentrated alcohol, hematoxylin and eosin stain powder, blood lancets, and a VEGF ELISA Kit.

The fruit of Sunkist were collected from the fruit market in Medan and the selection was based on the colour and quality of the peel. The identified Sunkist fruit was peeled to obtain the fruit peels extracted by maceration methods. 500 g Sunkist peel washed and dried to form a dry simplicial. After that, it was soaked in ethanol in a ratio of 1: 10 (simplicial: solvent) for five days and regularly stirred, then it was filtrated, and the residue was re-macerated two times for five days and evaporated at 70°C. Hence, it formed a concentrated form of extract.[8]

The evaluation of phytochemical compounds such as alkaloid, tannin, saponin, triterpenoid, steroid, flavonoid, glycone, aglycone, anthraquinone, and polyphenol was done by GC-MS spectroscopic detection with 70 eV ionization energy.[10] The amount of 0.5-gram sodium carboxymethyl cellulose was separated into the surface of warm distilled water (50 ml). After this mixture formed a clear semi-solid preparation, it was ground until it became homogenous. Then, the remaining distilled water (50 ml) was poured and stirred into this mixture, forming 0.5% Sodium carboxymethyl cellulose. Additionally, 1 gram of the concentrated extract was suspended in 0.5% sodium carboxymethyl cellulose to create an oral extract suspension with a concentration of 10 mg/mL. On the other hand, 0.5% Sodium carboxymethyl cellulose was used to make an oral metformin suspension by suspending 100 mg meshed metformin tablet into 5 ml 0.5% Sodium carboxymethyl cellulose, and it formed an oral suspension with a concentration of 20 mg/mL. All rats were induced by a single intraperitoneal injection of Streptozotocin solution at a dose of 50 mg/ kg BW. The streptozotocin powder was dissolved into 0.1 M citrate buffer (pH=4.5). However, before the injection, all rats were measured fasting blood glucose levels by a glucometer (10-12 hours fasting) that used a blood sample from a vein in the tail, and the fasting blood glucose level was also measured three days after the injection.[11] All rats with blood glucose levels higher than 200 mg/dL were selected as diabetic rats.[12] These diabetic rats were grouped into five groups: control, standard, intervention of Sunkist peels ethanol extract I, II, and III. The Control group received 1 ml 0.5% sodium carboxymethyl cellulose, and the standard group received metformin as a standard drug. Meanwhile, Sunkist peels ethanol extract I, II, and III groups received 500 mg/kgBW (0.5 mL/kgBW), 750 mg/kgBW (0.75 mL/ kgBW), and 1,500 mg/kg BW (1.5 mL/ kgBW) of Sunkist peels ethanol extract, respectively. It was given once a day in every morning for 28 days.[9,13]After 28 days, all rats were sacrificed by chloroform inhalation in a closed room. After that, all rats were fixed into paraffin blocks, and the abdomen wall was incised vertically to expose the organ in the abdomen cavity. Then, the kidney was dissected and washed into normal saline. Moreover, the washed kidney organ was kept in 10% buffer formalin solution (BFS) until it was processed to stain. The incision was also performed in the thorax of the rat for blood withdrawal. The blood

was collected by cardiac puncture using a 5 ml syringe and 25-G Needle. The blood was saved in red-coloured blood tube and centrifuged at 2,500 RPM for 10 minutes. After the centrifugation, the blood was separated, and the upper layer was collected into the microtube to undergo VEGF Serum measurement.[7,14] The obtained kidney tissue was sliced with a thick of 4–6 mm, and then it was dehydrated by alcohol in various concentrations (70%, 80%, 90%, and 95%) for 24 hours and in concentrated alcohol (100%) for an hour with three repetitions. The dehydrated tissue was washed with xylol for an hour with three repetitions. Moreover, it was washed with xylol for an hour with three repetitions. Then, it was infiltrated into a paraffin block to be dissected with a thickness of 4–5 µm. Finally, it was put into the object glass and stained with Haematoxylin-Eosin (HE). The damage to the kidneys was assessed based on changes in the histopathological structure, including tubular degeneration, albuminous degeneration, and tubular cell necrosis. In addition to kidney analysis, serum was also examined. The serum was analyzed to measure VEGF levels using the Enzyme-Linked Immunoassay (ELISA) method with an R&D Systems (Minneapolis, MN, USA) kit, and results were expressed in pg/ml.

The VEGF serum levels were analyzed using both descriptive and inferential statistics. Data distribution was first assessed using the Shapiro-Wilk test. If the data were normally distributed, one-way ANOVA was used for analysis; otherwise, Kruskal-Walli's analysis was performed. Data are presented as mean ± standard deviation, with statistical significance set at $p < 0.05$.

Results

The study identified that Sunkist as Kingdom Plantae, Division Spermatophyta, Class Dicotyledoneae, Ordo Sapindales, Family Rutaceae, Genus Citrus Species, Citrus sinensis (L.) Osbeck. Based on the result of identification, this study used Sunkist Peels with the scientific name of Citrus sinensis (L.) Based on the identification results, this study used Sunkist peels, scientifically known as Citrus sinensis (L.) Osbeck, which belongs to the Rutaceae family. Maceration was employed to extract the peels. The resulting extract contained various phytochemicals, including tannins, saponins, flavonoids, triterpenoids, steroids, and polyphenols.

Table 1 showed that there were ten main compounds, that were identified from GC-MS analysis of Sunkist peel Ethanol extract. These compounds were 2-Bromoethanol (1.82%) 4H-Pyran-4-One,2,3-Dihydro-3,5-Dihyroxyl-6-Methyl (4.43%), 2-Furancarboxaldehyde-5-(Hydromethyl)-(15.58%),2-Methoxy-4-Vinylphenol (1.84%), Alpha-Aminooxy-Propionic acid, ethyl ester (1.72%), Ethyl-α-d-glucopyranoside (20.61%), 3-Deoxy-d-Mannonic Acid (25.10%), Allo-Inositol (23.84%), Methyl [Methyl 5-Acetamido-7-O-Acetyl- 3,4,5-Trideocy-4-C-Methyl-8,9-O-(Methylethylidene)-

Table 1 GC-MS Analysis of the Extract

RT	Quality	Compounds	Concentration (%)
5.393	38	2-Bromoethanol	1.82
7.221	94	4H-Pyran-4-One, 2, 3-Dihydro-3,5-Dihyroxyl-6-Methyl	4.43
10.972	87	2-Furancarboxaldehyde-5-(Hydromethyl)-	15.58
11.758	49	2-Methoxy-4-Vinylphenol	1.84
22.804	46	Alpha-Aminooxy-Propionic acid, ethyl ester	1.72
27.838	76	Ethyl-α-d-glucopyranoside	20.61
28.927	64	3-Deoxy-d-Mannonic Acid	25.10
31.051	47	Allo-Inositol	23.84
39.794	47	Methyl [Methyl 5-Acetamido-7-O-Acetyl-3,4,5-Trideocy-4-C-Methyl-8,9-O-(Methylethylidene)-β-D-Glycerol-D-Glycerol-D-Galacto-2-Nonulopyranosid] Onate	1.47
40.284	78	2-(3,4-Dimethoxyphenyl)-5,6,7,8-Tetramethoxy-4H-1-Benzopyran-4-One	2.46

*RT: Retention Time

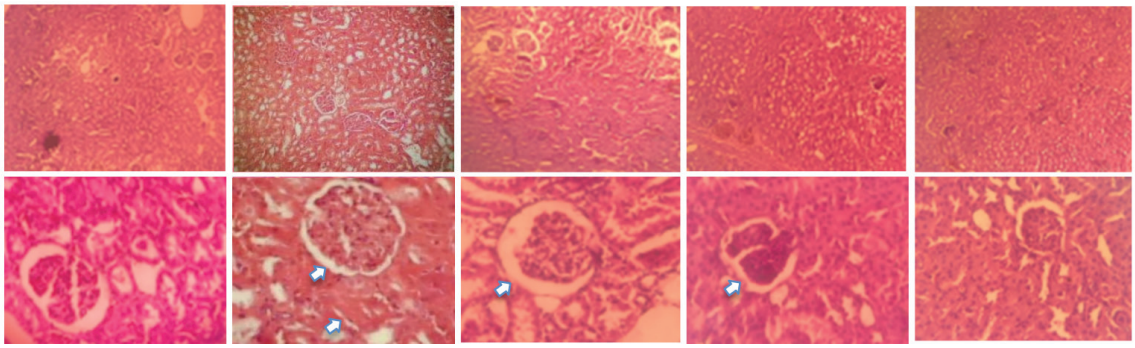

Figure 1 Histology of Kidney. Stain: Hematoxylin & Eosin (HE). Magnification: 10x (Upper) and 40x (Lower)

D-Glycerol-D-Glycerol-D-Galacto-2-Nonulopyranoside] Onate (1.47%), and 2-(3,4-Dimethoxyphenyl) -5,6,7,8- Tetramethoxy-4H-1-Benzopyran-4- One (2.46%).

The Sunkist peel extract was utilized for in vivo assays to assess its nephroprotective effects in a diabetic nephropathy rat model. The in vivo assays included histological examination of kidney tissues and measurement of VEGF levels to evaluate the nephroprotective impact of the Sunkist peel ethanol extract. The results of the histological analysis of kidney tissue are presented in Figure 1.

Based on Figure 1, it is obvious that the normal structure of the kidney was observed in the standard group, without any damage in pathologic. Serious damage was found in the control group. It showed a wide necrosis in the tubular structure with the foci of hemorrhage. Meanwhile, the other groups that received various doses of Sunkist peel extract also revealed various degrees of kidney tissue damage. At the lowest dose (Sunkist Peel Ethanol Extract-I), the kidney showed a glomerulus shrinkage and vacuolated tubular tissue. At the higher dose (Sunkist Peel Ethanol Extract-II), the kidney tissue revealed no vacuolated tubular structure like in the lowest dose group. However, the shrinkage of the glomerular structure was still found in the lowest dose group. At last, the highest dose group revealed less kidney pathology damage, which only revealed degeneration of tubular structure without any glomerular damage. Furthermore, this study not only performed a histology study against the kidney tissue but also evaluated VEGF level as the inflammatory marker to support the histology study against the kidney tissue.

The inflammatory marker VEGF level was assessed to evaluate the severity of microvascular damage, including diabetic nephropathy, and to complement the histological study. Initially, the data distribution of VEGF levels was analyzed using the Shapiro-Wilk test, which revealed that the VEGF levels in the control and Sunkist peel ethanol extract-III groups were not normally distributed (p-value<0.05). Consequently, data analysis was continued with the Kruskal-Wallis test.

The VEGF levels revealed that there was a significant difference between the groups (p-value<0.05. Moreover, it can be seen that the increased dose of Sunkist peels extracts significantly followed by the increase of VEGF level. The highest VEGF level found in the control group was 275.57 mg/kg, and the lowest was

Table 2 VEGF Levels of All Treatment Groups

Groups	VEGF Level (mg/kg)	p-value
Control	275.57 (274.56–299.10)	
Standard	265.50 (235.55–291.03)	
Extract-I	245.62 (235.21–246.99)	<0.05
Extract-II	187.90 (159.17–195.75)	
Extract-III	127.58 (111.34–185.60)	

found in the Sunkist Peel Ethanol Extract-III, that was 127.58 mg/kg.

Discussion

This study answered the aims of investigating the nephroprotective effect of Sunkist peel extract against the diabetic nephropathy rat model. The Sunkist peels may protect the macroscopic structure of the kidney from diabetic nephropathy. The improvement histology view from kidney tissue among the rats that received the Sunkist peels extract can be seen from the improvement histology view. This improvement was also followed by decreases in VEGF levels, which act as inflammatory markers in this study. Furthermore, it showed the increase of Sunkist Peels Ethanol Extract also increased the nephroprotective effect against the diabetic nephropathy that Streptozotocin induced.

Streptozotocin is a cytotoxic glucose analog compound that may inhibit DNA synthesis in bacterial and mammalian cells. A prolonged hyperglycaemic state or uncontrolled diabetes may cause significant kidney damage. This damage can trigger abnormalities in kidney function and histological structure, including accumulation of extracellular matrix, thickening of the glomerular membrane, and glomerular sclerosis.[16,17] Kidney damage was also assessed based on the degeneration of tubular structures. Streptozotocin induces kidney damage, leading to degeneration and necrosis of both proximal and distal tubular epithelium.[18] Diabetic nephropathy can cause several pathology and clinical changes. These changes include thickening of the glomerular basement membrane (GBM), diffuse or nodular glomerulosclerosis, arteriolar hyalinosis, tubulointerstitial fibrosis, and increased albumin excretion in the urine (or albuminuria). Albuminuria can occur due to the increased permeability of the glomerular filtration barrier to albumin and various protein plasma.[5,19] This change in permeability may be characterized by VEGF, a potent inducer of microvascular permeability. In the diabetic state, upregulation of VEGF and/or its signaling activity in the glomerulus can lead to albumin leakage from the glomerular filtration barrier.Moreover, VEGF also causes nitric oxide-mediated vasodilation and contributes to albuminuria in diabetic settings. Thus, it was obvious that a decrease in VEGF Level followed the increase of the Sunkist Peels ethanol extract, which means the increase of Sunkist peel ethanol extract may reduce the severity of albuminuria and improve the kidney function in the nephropathy diabetic.[18,20]. The previous study with the same methods, the previous study evaluated different parameters that were BUN and Creatinine levels. The previous study demonstrates that the Sunkist peels extract may protect rat kidneys from diabetic nephropathy.[9]

The nephroprotective effect of Sunkist peel ethanol extract was associated with the presence of the various phenolic compounds including phenol, flavonoid, and tannin. The previous study found the total phenolic, tannin, and flavonoid content were 9.89±0.19 mg GAE/ g extract, 44.27±0.29 mg TAE/ g extract, and 3.83±0.00 mg QE/ g extract. Another study performed by Gulo et al. with two different solvents also reported that the total flavonoid content from sweet orange peel ethanol and ethyl acetate was 205.66±49.63 and 242.48±13.83 mg QE/ gr DW, respectively. However, the previous study only reported the total phenolic compounds contents but did not report the obvious content of these compounds. Thus, the recent study continued to analysed these compounds by GC-MS analysis and the GC-MS detected some phenolic compounds like 2-Bromoethanol (1.82%) 4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihyroxyl-6-Methyl (4.43%), 2-Furancarboxaldehyde-5-(Hydromethyl)-(15.58%), 2-Methoxy-4-Vinylphenol (1.84%), Alpha-Aminooxy-Propionic acid, ethyl ester (1.72%), Ethyl-α-d-glucopyranoside (20.61%), 3-Deoxy-d-Mannonic Acid (25.10%), Allo-Inositol (23.84%), Methyl [Methyl 5- Acetamido- 7- O-Acetyl- 3, 4, 5-Trideocy- 4-C-Methyl-8,9-O-(Methylethylidene)-D-Glycerol-D- Glycerol-D-Galacto-2-Nonulopyranoside] Onate (1.47%), and 2-(3,4-Dimethoxyphenyl)-5,6,7,8-Tetramethoxy- 4H-1-Benzopyran-4-One (2.46%). [6,21]

The results of the GC-MS analysis in the current study are consistent with those reported by Ahmed et al.,[22] who found that the hydroethanolic extract of navel orange peels contains various phytochemicals with potent biological activities. These compounds included 4H-Pyran-4-One, Thymine, 5-Hydroxymethylfurfural and 4-hexen-3-one,4,5-dimethyl-, Dodecane, 2-Methoxy-4-vinylphenol, 3-methoxyacetophenone, 9,12-Octadecadienoic acid, 3-methoxyacetophenone, Oleic acid, 9-octadecenoic acid, Lupanine, Stigmasterol, and 9-Octadecenamide. Moreover, this study also described the potential biological activities of these compounds. Other compounds that

also found in the Sunkist peels extract in the recent study were 2-Methoxy-4-Vinylphenol and 4H-Pyran-4-One. 4H-Pyran-4-One, detected at a retention time of 7.221 and a concentration of 4.43%, it has various biological activities, including hepatoprotective effect, anti-oxidant, anti-bacterial, antiviral, anti-cancer, and anti-inflammatory effects. In the recent study, 4H-Pyran-4-One was found as the 4H-Pyran-4-One, 2, 3-Dihydro-3,5-Dihyroxyl-6-Methyl formed a complex compound with the 2,3-Dihydro-3,5- Dihyroxyl-6-Methyl and this compound was indicated have a potent free radical scavenging activity. Another compound found in the extract was 2-Methoxy-4-vinylphenol, which was detected at a retention time of 11.758 and a concentration of 1.84%. This compound is known to exhibit several biological activities, including antioxidant, antimicrobial, and anti-inflammatory effects. Additionally, a study conducted by Suhartomi et al. demonstrated that various fractions of orange peels, including methanol, n-hexane, and dichloromethane, possess antioxidant activity, specifically hydrogen peroxide and DPPH scavenging activities. Therefore, these compounds contribute to the nephroprotective effects of Sunkist peel ethanol extract through their antioxidant and anti-inflammatory properties. [6,22]

Overall, the findings indicate that Sunkist peel extract has potential nephroprotective effects against diabetic nephropathy, primarily through its antioxidant and anti-inflammatory properties. This results in reduced injury to kidney tubular cells, as evidenced by a decrease in the average severity of renal tubular degeneration and necrosis. However, the study has limitations; specifically, it did not measure the Glomerular Filtration Rate (GFR). Future research should include GFR assessment to better evaluate the impact of Sunkist peel extract on the physiological functions of the glomeruli.

References

1. Zhou B, Carrillo-Larco RM, Danaei G, et al. Worldwide trends in hypertension prevalence and progress in treatment and control from 1990 to 2019: a pooled analysis of 1201 population-representative studies with 104 million participants. Lancet. 2021;398(10304):957–80. doi:10.1016/S0140-6736(21)01330-1
2. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract. 2019;157:107843. doi:10.1016/j.diabres.2019.107843
3. Syafril S. Pathophysiology diabetic foot ulcer. IOP Conf Ser Earth Environ Sci. 2018;125(1):012161. doi:10.1088/1755-1315/125/1/012161
4. Selby NM, Taal MW. An updated overview of diabetic nephropathy: diagnosis, prognosis, treatment goals and latest guidelines. Diabetes, Obes Metab. 2020;22(S1):3–15. doi:10.1111/dom.14007
5. Sagoo MK, Gnudi L. Diabetic nephropathy: an overview. Diabet Nephrop Methods Protoc. Published online 2020:3-7.
6. Suhartomi, Gulo KN, Saragih AD, Martinus AR, Ikhtiari R. Antioxidant properties of sweet orange peels in several fractions of methanolic extract. ICHIMAT. Scitepress Digital Library; 2020:371–78. doi:10.5220/0009515503710378
7. Depari SAF, Rambe DJA, Meilando R, Lisya C, Mutia MS, Lubis YEP. Uji efektivitas ekstrak etanol kulit jeruk sunkist (citrus sinensis (L.) Osbeck) terhadap kadar gula darah tikus wistar (rattus norvegicus) dengan hiperkolesterolemiai yang diinduksi Streptozotocin. Biospecies. 2021;14(1):1–9.
8. Mutia MS, Chiuman L. Hepatoprotective Effect of citrus sinensis (L.) osbeck ethanol extract in paracetamol-induced rats. Majalah Kedokteran Bandung. 2019;51(4):189–93. doi:10.15395/mkb.v51n4.1794
9. Sari M, Ginting CN, Yulizal O. Sunkist peel ethanol extract ameliorates diabetic nephropathy in streptozotocin-induced diabetic wistar rats. Open Access Maced J Med Sci. 2021;9(A):1086–91. doi:10.3889/oamjms.2021.7362
10. Sabina E, Zaidul ISM, Ghafoor K, Jaffri JM, Sahena F, Babiker EE, et al. Screening of Various Parts of Phaleria macrocarpa Plant for α-Glucosidase Inhibitory Activity. J Food Biochem. 2016;40(2):201–10. doi:10.1111/jfbc.12212
11. Bayir MH, Yıldızhan K, Altındağ F. Effect of hesperidin on sciatic nerve damage in stz-induced diabetic neuropathy: modulation of TRPM2 channel. Neurotox Res. 2023;41(6):638–47. doi:10.1007/s12640-023-00657-0
12. Wardana AP, Tukiran. Skrining fitokimia

dan aktivitas antioksidan ekstrak kloroform tumbuhan gowok (syzygium polycephalum). Pros Semin Nas Kim dan Pembelajarannya. 2016;(September):1-6.
13. Nagaraja S, Ahmed SS, Bharathi DR, Goudanavar P, Rupesh KM, Fattepur S, et al. Green synthesis and characterization of silver nanoparticles of psidium guajava leaf extract and evaluation for its antidiabetic activity. molecules. 2022;27(14):4336. doi:10.3390/molecules27144336
14. Li B, Wang H, Zhou G, et al. VEGF-loaded biomimetic scaffolds: a promising approach to improve angiogenesis and osteogenesis in an ischemic environment. RSC Adv. 2017;7(8):4253–9. doi:10.1039/C6RA25294J
15. Guo L, Jiang F, Tang YT, Si MY, Jiao XY. The association of serum vascular endothelial growth factor and ferritin in diabetic microvascular disease. Diabetes Technol Ther. 2014;16(4):224–34. doi:10.1089/dia.2013.0181
16. Worotikan R V., Tuju EA, Kawuwung F. Analisa efektivitas antidiabetes ekstrak etanol buah andaliman (zanthoxylum acanthopodium DC) pada histopatologi ginjal tikus putih (rattus norvergicus) yang diinduksi alloksan [analysis of antidiabetic effectiveness of lemon pepper fruit (zanthoxylum aca. J Sains Mat Edukasi. 2017;5(1):29–37.
17. Alotaibi MR, Fatani AJ, Almnaizel AT, Ahmed MM, Abuohashish HM, Al-Rejaie SS. In vivo assessment of combined effects of glibenclamide and losartan in diabetic Rats. Med Princ Pract. 2019;28(2):178–85. doi:10.1159/000496104
18. Chen S, Ziyadeh FN. Vascular endothelial growth factor and diabetic nephropathy. Curr Diab Rep. 2008;8(6):470–6. doi:10.1007/s11892-008-0081-3
19. Gnudi L, Long DA. Diabetic nephropathy: methods and protocols. Springer; 2020.
20. Chyla G, Slaaga-Zaleska K, Dabkowski K, Kuchta A, Jankowski M. Suramin Enhances the Urinary Excretion of VEGF-A in Normoglycemic and Streptozotocin-Induced Diabetic Rats. Pharmacol Reportd. 2021;73:841–6.
21. Gulo KN, Suhartomi, Saragih AD, Raif MA, Ikhtiari R. Antioxidant activity of flavonoid compounds in ethanol and ethyl acetate extract from citrus sinensis. In: AIMS 2021-International Conference on Artificial Intelligence and Mechatronics Systems. IEEE; 2021:1–6. doi:10.1109/AIMS52415.2021.9466078
22. Ahmed OM, Fahim HI, Ahmed HY, et al. The preventive effects and the mechanisms of action of navel orange peel hydroethanolic extract, naringin, and naringenin in n-acetyl-p-aminophenol-induced liver injury in wistar rats. Oxid Med Cell Longev. 2019;2019:1–19. doi:10.1155/2019/2745352

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Effectiveness of Short-Term Use Denosumab and Risedronate Using β-Crosslaps and Histopathology as a Parameter in Osteoporotic Rat Model

Afrisya Bimo Siwendro,[1] Ahmad Ramdan,[1] Yoyos Dias Ismiarto,[1] Anglita Yantisetiasti,[2] Dliyauddin Fachri[1]

[1]Department of Orthopaedic and Traumatology, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital Bandung, Indonesia

[2]Department of Anatomical Pathology, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital Bandung, Indonesia

Abstract

Osteoporosis is a condition of decreased bone mass density. Pharmacological management uses drugs that decrease bone resorption or increase bone formation. The most commonly used drugs are bisphosphonates. Risedronate is one of these bisphosphonates. It inhibits osteoclasts, resulting in osteoclast apoptosis. Denosumab is a human monoclonal antibody that inhibits receptor activator kappa-B ligand, inhibiting osteoclast activation. Both drugs are widely used. Research on the efficacy of these two mechanisms has yet to obtain conclusive results. β-cross-laps is a parameter that can be used to evaluate the effectiveness of therapy through monitoring the bone resorption process. This experimental study used female rats >9 weeks old and was conducted at the Pharmacology and Therapeutic Laboratory, Universitas Padjadjaran Bandung, Indonesia, from June to September 2021. This study utilized a simple random sampling to allocate 24 experimental animals into three groups: control, risedronate, and denosumab. β-Crosslaps expression values before ovariectomy, post ovariectomy before receiving medication, and post ovariectomy and receiving medication was recorded and statistically analyzed using the SPSS version 24.0. The analysis of 24 samples revealed a statistically significant decrease in the median value of β-Crosslaps after ovariectomy in the denosumab group (p=0.036) when compared to the control group, whereas the decrease in the risedronate group was not significant (p=0.687). Administration of denosumab in rat models is more effective in reducing bone resorption compared to risedronate.

Keywords: β-cross-laps, denosumab, risedronate, osteoporosis

Introduction

Osteoporosis is a public health problem that will increase as the population ages. Osteoporosis is a condition of decreasing bone mass density (BMD) to the reference. According to WHO, osteoporosis is when bone mass density has a T-score < –2.5; normal if the T-score > -1 and osteopenia if the T-score is between -1 to -2.5. [1]

Osteoporosis is caused by a combination of excess bone structure resorption, inadequate bone formation, and an imbalance in activity between bone cells that play a role in the remodeling process.[2] Osteoporosis occurs as a result of an increase in the number and activity of osteoclasts, the cells responsible for bone resorption; decreased number and activity of osteoblasts, bone-forming cells; or the presence of areas of bone that show both characteristics of abnormal bone cells.[2]

Bone mineral density (BMD) is the most important predictor for predicting fracture risk in osteoporosis. There are various methods available to evaluate bone loss. β-Crosslaps is a C-terminal telopeptide of type I collagen, a major component of the protein matrix of bone. β-Crosslaps are released into the bloodstream during resorption and are almost entirely excreted via the kidneys. Its level can be a specific parameter for the degradation of mature type I

Corresponding Author:
Yoyos Dias Ismiarto
Department of Orthopaedic and Traumatology, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital Bandung, Indonesia
Email: yoyosismiartounpad@gmail.com

collagen from the bone.[3]

Pharmacological management of osteoporosis focuses on targeted remodeling through drugs that decrease bone resorption.[3] Bisphosphonates belong to the group of antiresorptive agents that have an inhibitory effect on osteoclasts, which reduces bone resorption.[4] Risedronate can inhibit bone resorption by reducing the action of the enzyme farnesyl pyrophosphate synthase (FPPS) at the osteoclastic level. Risedronate is also widely distributed in bone and has a half-life of up to weeks. Risedronate can reduce bone resorption by up to 50%.[5]

Denosumab is a human monoclonal antibody that inhibits the receptor activator kappa-B Ligand (RANKL) from binding to osteoprotegerin (OPG) so that the process of inhibiting osteoclast activation occurs. Denosumab has specific properties on certain TNF groups, including TNFa, TNF, and TNF associated with apoptosis. Denosumab is used for the management of metabolic bone diseases, including postmenopausal osteoporosis and osteoporosis due to glucocorticoid consumption.[6] Both risedronate and denosumab have the goal of reducing bone resorption. Research on the efficacy has yet to obtain conclusive results, so further research is still needed. In addition, no studies have been found that examine the effectiveness of denosumab with risedronate, which was assessed histopathologically. Thus, based on the description above, the authors conducted a study comparing the effectiveness of the short-term use of denosumab with risedronate using β-Crosslaps and histopathology parameters in a rat model of osteoporosis.[7]

Methods

This study is an animal experimental research with a parallel design and a double-masked method, aimed at investigating causal correlations using experimental animals. The research received approval from the Health Research Ethics Committee of Universitas Padjadjaran (No. 683/UN6.KEP/EC/2021). It was conducted at the Pharmacology and Therapeutic Laboratory, Faculty of Medicine, Universitas Padjadjaran, Bandung, from June to September 2021. The study utilized female rats (*Mus musculus L.*) aged over nine weeks. Sample size calculations using the Federer formula determined that 8 samples per group were required.

Inclusion criteria for this study were female rats aged over nine weeks, weighing between 250 and 290 grams, in good health, and having undergone ovariectomy and dexamethasone injection to induce osteoporosis. Exclusion criteria included noticeable behavioral changes in the rats, such as refusal to eat or lack of movement. Dropout criteria comprised rats that died during the adaptation period or throughout the experiment.

The materials used in this study included experimental animals, risedronate tablets, denosumab injection, carboxymethyl cellulose powder, aquabides, dexamethasone injection, ketamine for surgery, a mouse β-Crosslaps ELISA kit, and food and water for the rats. The equipment required included animal cages, a minor surgical instrument set, a nasogastric tube, a 1 cc syringe, a plain vacuum tube, and general rat rearing equipment.

Experimental rats were obtained from the Pharmacology Laboratory of Universitas Padjadjaran. The rats underwent a one-week adaptation and conditioning period prior to the study. All rats were subjected to ovariectomy and received dexamethasone injections (0.1 mg/kg body weight, subcutaneously) to induce osteoporosis.

Randomization was achieved by dividing 18 rats into two initial groups of nine rats each. An additional group of nine rats, which did not receive any treatment, was included. This resulted in three groups: the control group (G1), the Risedronate group (G2), and the Denosumab group (G3). Rats in the control group (G1) did not receive any treatment for osteoporosis after the induction phase. Rats in the Risedronate group (G2) were administered risedronate orally at a dosage of 0.24 mg/kg body weight once daily for four weeks. Concurrently, rats in the Denosumab group (G3) received Denosumab injections subcutaneously at a dose of 6 mg/kg weekly for four weeks.

Blood samples will be drawn three times before ovariectomy, one month after ovariectomy and administration of dexamethasone, and one month after administration of denosumab or risedronate. The first and second samples were drawn from the tail blood/serum using a one cc syringe, while the third sample was taken from the heart after termination. Each blood draw is inserted into a vacuum tube (plain), recording the time from the start of inserting the blood into the tube. The sampling tube is a disposable, non-pyrogenic, and non-endotoxin tube. Next, the sample is sent from the Pharmacology Laboratory to the Clinical Pathology Laboratory using a bag/box at room.

The termination of the animals was carried out anesthetically using Pentobarbital, administered intravenously at a dosage of 100-150 mg/kg b.w. temperature and stored at -80°C. After all the samples were collected, they were stored at -80°C. Once ready for analysis, the samples were removed from the freezer and allowed to reach room temperature before being examined with the β-Crosslaps ELISA kit. The β-Crosslaps method uses a competitive ELISA to quantify C-terminal telopeptides of type I collagen (CTx-I), markers of bone resorption. Serum or plasma is introduced to antibody-coated wells, followed by a secondary, enzyme-linked antibody that competes with CTx-I for binding. The resulting color intensity from a chromogenic substrate is inversely related to CTx-I levels, providing a measure of bone turnover, crucial for diagnosing related conditions. Histopathological examination was carried out after euthanasia in rats using an anesthetic agent. The fragments of the femur bone were taken and then washed using 70% alcohol, purified with butyl alcohol (n-butanol), and added to paraffin. After that, axial and vertical sections with a thickness of 5 mm were taken and stained using the modified Masson Goldner's Trichome method and examined under an optical microscope to see bone components such as the haversian system and bone structure.31 Histopathological assessment was assessed by assessing the volume of the trabeculae bone as follows: 0= none, 1= mild, 2=moderate, 3=severe.

Statistical analysis was conducted to evaluate the significance of each variable using the Mann-Whitney test, performed with SPSS version 23.

Results

The study was carried out from June to September 2021. Statistical test results in the research group on variable post-ovariectomy results, as shown in Table 1, obtained a p-value of 0.052 which is considered marginally significant. Post hoc analysis was continued to determine the more effective mechanism of action between risedronate and denosumab. In addition, it can be explained that there is no statistically significant β-Crosslaps difference between the three groups post-ovariectomy. The absence of statistically significant differences in β-Crosslaps levels among the three groups post-ovariectomy suggests effective therapeutic intervention. β-Crosslaps, a marker of bone resorption, typically increases following ovariectomy due to the resultant estrogen deficiency, which accelerates bone turnover. However, the introduction of treatments such as risedronate and denosumab in the study groups appears to have mitigated this effect.

The β-Crosslaps levels measured after ovariectomy in the control group, compared to those in the risedronate group, yielded a p-value of 0.052, which is marginally significant. The median β-Crosslaps values were 100.05 and 77.80, respectively. This suggests a decrease in β-Crosslaps in the risedronate group, indicating reduced bone resorption, although the result does not reach statistical significance under the strict $p<0.05$ criterion.

In contrast, the comparison between the control group (G1) and the denosumab group (G3) produced a p-value of 0.038, indicating a statistically significant difference. The median β-Crosslaps values for these groups were 100.05 and 70.75, respectively. This demonstrates a significant reduction in β-Crosslaps in the denosumab group, reflecting a reduction in bone resorption that is statistically significant.

Meanwhile, the group given risedronate and denosumab showed a p-value greater than 0.05 ($p>0.05$), indicating no statistically significant difference between these two treatment groups. .Based on a repeated ANOVA analysis with a p-value of 0.027 in the control group (G1), there is a statistically significant difference between Outcome II and III. The median β-Crosslaps

Table 1 Comparison Results of β-Crosslaps (ng/mL) After Ovariectomy Outcome III In Three Groups

Variable	n	Median	Range (min.-max.)	p value
G1	8	100.05	62.90-148.80	
G2	8	77.80	62.60-112.10	**0.052****
G3	8	70.75	51.50-120.70	

Description: Numerical data was tested with the Kruskal Wallis test—the significance value is based on $p<0.05$. The * sign indicates the p value<0.05 means significant or statistically significant. The ** sign indicates the value for marginally significant. G1= control group; G2=Group receiving risedronate; G3=Group receiving denosumab

Table 3 Comparison of Changes in β-Crosslaps Results Between Outcome II and III

Group	n	Outcome (ng/mL)		p value
		II	III	
G1	8			
Median		76.95	100.05	**0.027***
Range (min-max)		61.10-93.50	62.90-148.80	
G2	8			
Median		74.05	77.80	**0.687**
Range (min-max)		38.10-85.70	62.60-112.10	
G3	8			
Median		77.80	70.75	**0.036***
Range (min-max)		61.40-88.60	51.50-120.70	

Description: The Repeated Anova test tested numerical data of p-value. The value of significance is based on the p-value of <0.05. The * sign indicates the p value<0.05 means significant or statistically significant. G1= control group; G2=The group that received risedronate; G3 = Group receiving denosumab. Outcome I = before ovariectomy, Outcome II = after ovariectomy, before medication is given, Outcome III = after ovariectomy, after medication

value increased from 76.95 pre-medication to 100.05 post-medication, indicating successful osteoporosis induction through the immobilization process during the two-month maintenance period in the treatment cages.

Comparing post-ovariectomy β-Crosslaps values before medication between the control group (G1) and the group given risedronate (G2) shows median values of 76.96 and 74.05, respectively. With a p-value greater than 0.05 (p>0.05), this indicates no statistically significant difference between these groups' pre-medication, suggesting that while risedronate inhibits bone resorption, its effect was not significant enough to differ statistically from the control.

The results in the group given denosumab (G3) revealed a p-value of 0.036, indicating a statistically significant decrease in bone resorption, consistent with findings from a 2012 study by McClung et al., which documented a persistent increase in bone mineral density (BMD) in the lumbar spine and hip over 8 years with denosumab treatment, resulting in final increases of 16.5% and 6.8%, respectively.[8]

Table 3 presents the median and range values for β-Crosslaps at two different time points: Outcome II (prior to medication) and Outcome III (after medication). In the Control group (G1), the median β-Crosslaps levels increased from 76.95 ng/mL at Outcome II to 100.05 ng/mL at Outcome III. The range also widened from 61.10-93.50 ng/mL to 62.90-148.80 ng/mL. This change was statistically significant, with a p-value of 0.027*, indicating a rise in β-Crosslaps levels following ovariectomy without any pharmacological intervention.

In the Risedronate group (G2) the β-Crosslaps levels showed a minor increase from a median of 74.05 ng/mL at Outcome II to 77.80 ng/mL at Outcome III, with the range extending from 38.10-85.70 ng/mL to 62.60-112.10 ng/mL. This increase was not statistically significant, as reflected by a p-value of 0.687.

In contrast, the Denosumab group (G3) exhibited a decrease in median β-Crosslaps levels from 77.80 ng/mL at Outcome II to 70.75 ng/mL at Outcome III. The range varied from 61.40-88.60 ng/mL to 51.50-120.70 ng/mL. This reduction was statistically significant, with a p-value of 0.036*.

Discussion

This study evaluated the effects of short-term administration of denosumab and risedronate on β-Crosslaps levels and histopathological changes in an experimental osteoporosis model. The data indicate that neither denosumab nor risedronate demonstrated a significant advantage over the other in reducing β-Crosslaps levels over three months. However, denosumab exhibited a slightly more pronounced effect in decreasing bone resorption markers compared to risedronate, as evidenced by changes observed from pre-medication to post-medication phases. These findings suggest the need for further research comparing short-term and long-term pharmacological interventions for osteoporosis treatment. Extended observation periods are necessary to fully assess the therapeutic potential and histological impact of these drugs.

In another study conducted by Saag et al. where a comparison of the administration of risedronate with denosumab resulted in a significantly more significant increase in BMD in the femoral neck, lumbar spine, and total hip with denosumab than with risedronate as measured at 12 months of follow-up and 24 months.[9] The same study also reported the results of denosumab administration were compared with risedronate on bone resorption markers, assessed by the CTX and P1NP markers showing that denosumab was superior in increasing bone mineral density (BMD) at all assessed time points.

Previous studies have typically evaluated the effects of denosumab and risedronate over extended periods (greater than one year), with limited research focusing on short-term administration. For instance, a study by Murat Arslan et al. compared the effects of bisphosphonates administered for six months and found that β-Crosslaps levels had a significance value of 0.034, indicating a significant reduction compared to treatments involving hormones.[1]

Histopathological results showed no significant difference in the number of osteoclasts, osteoblastic rimming, trabecular thickness, lamellar meshwork density, and bone matrix percentage between the control group and groups receiving risedronate or denosumab. These findings suggest that short-term administration does not significantly alter these histological features, which is consistent with the β-Crosslaps results shown in Tables 2 and 3, indicating similar efficacy in bone resorption reduction between risedronate and denosumab. However, these results of this study differ from the study conducted by David et al. in 2018, which showed that the biopsy results of patients given denosumab for ten years showed normal histology.[9]

A study in the United States in 2010 showed that Denosumab significantly reduced bone resorption and reduced fracture rates. The results of the qualitative histological evaluation of the biopsy showed no significant results. In the Freedom study, median eroded surface was reduced by more than 80% and osteoclasts did not there were more than 50% of the biopsy results in the denosumab group.[10] The mean bone formation rate was reduced by 97%.42 The McClung et al. study, also demonstrated that denosumab significantly improved the trabecular bone score in postmenopausal women with osteoporosis. The percentage change in FFB was statistically significant compared to baseline ($p < 0.001$) and placebo (p 0.014). [11]

The results of previous studies differ from this study because there are differences in the time of the intervention. In this study, denosumab and risedronate were given for 3 months, whereas in the previous study, the average denosumab was given for 3 years every six months, and risedronate was given for 1 year every 6 months. The results of this study, as summarized in Table 2, indicate that denosumab and risedronate did not show a significant difference in terms of β-Crosslaps levels, with both treatments demonstrating similar effectiveness in reducing bone resorption ($p>0.05$). However, as shown in Table 3, denosumab was more effective in reducing β-Crosslaps levels from the pre-medication to post-medication phases compared to risedronate. This suggests that, although both treatments are similarly effective overall, denosumab may have a slightly superior effect on bone resorption in the short term. Further research with extended study periods is needed to fully assess the long-term effects of denosumab and risedronate on β-Crosslaps levels and histopathological features, as the short-term administration (less than one year) may not capture the complete effects of these treatments.

References

1. Aslan M, Çogendez E, Education ZK, Eken M, Education ZK, Arioglu P, et al. Serum beta crosslaps as a predictor for osteoporosis in postmenopausal women. İstanbul Tıp Fakültesi Dergisi. 2015;78:36–40. doi: 10.18017/iuitfd .m.13056441.2015.78/2.36-40
2. Suzuki T, Nakamura Y, Kato H. Calcium and vitamin D supplementation with 3-year denosumab treatment is beneficial to enhance bone mineral density in postmenopausal patients with osteoporosis and rheumatoid arthritis. Therapeutics and clinical risk management. 2019;15:15.
3. Golob AL, Laya MB. Osteoporosis: screening, prevention, and management. Medical Clinics. 2015;99(3):587–606.
4. Dima L. Diab, Nelson B. Watts, Paul D. Miller, Bisphosphonates pharmacology and use in the treatment of osteoporosis. In: David W. Dempster, Jane A. Cauley, Mary L. Bouxsein, Felicia Cosman, Marcus and Feldman's, Editors. Osteoporosis. 5th ed. Cambridge:

Academic Press; 2021; p. 1721–36.
5. Augoulea A, Tsakonas E, Triantafyllopoulos I, Rizos D, Armeni E, Tsoltos N, et al. Comparative effects of denosumab or bisphosphonate treatment on bone mineral density and calcium metabolism in postmenopausal women. J Musculoskelet Neuronal Interact. 2017;17(1):444–9.
6. Langdahl B, Ferrari S, Dempster DW. Bone modeling and remodeling: potential as therapeutic targets for the treatment of osteoporosis. Ther Adv Musculoskelet Dis. 2016;8(6):225–35. doi:10.1177/1759720X16670154
7. Saag KG, Pannacciulli N, Geusens P, Adachi JD, Messina OD, Morales-Torres J, et al. Denosumab versus risedronate in glucocorticoid-induced osteoporosis: final results of a twenty-four-month randomized, double-blind, double-dummy trial. Arthritis Rheumatol. 2019;71(7):1174–84. doi:10.1002/art.40874
8. Hu M, Zhang Y, Guo J, Guo C, Yang X, Ma X, et al. Meta-analysis of the effects of denosumab and romosozumab on bone mineral density and turnover markers in patients with osteoporosis. Front Endocrinol (Lausanne). 2023;14:1188969. doi:10.3389/fendo.2023.1188969
9. Dempster DW, Brown JP, Fahrleitner-Pammer A, Kendler D, Rizzo S, Valter I, et al. Effects of long-term denosumab on bone histomorphometry and mineralization in women with postmenopausal osteoporosis. J Clin Endocrinol Metab. 2018;103(7):2498–509.
10. Tourolle DC, Dempster DW, Ledoux C, Boaretti D, Aguilera M, Saleem N, et al. Ten-year simulation of the effects of denosumab on bone remodeling in human biopsies. JBMR Plus. 2021;5(6):e10494. doi:10.1002/jbm4.10494
11. McClung MR, Lippuner K, Brandi ML, Zanchetta JR, Bone HG, et al. Effect of denosumab on trabecular bone score in postmenopausal women with osteoporosis. Osteoporos Int. 2017;28(10):2967–73. doi:10.1007/s00198-017-4140-y

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Patient's Quality of Life After 3-and 6-Months Cataract Surgery

Pieter Juanarta, Feti Karfiati, Budiman
Department of Ophthalmology, Faculty of Medicine Universitas Padjadjaran
Cicendo National Eye Hospital, Bandung, Indonesia

Abstract

Cataract surgery is the most common elective procedure and has been shown to increase patients' visual acuity. However, visual acuity alone cannot adequately represent their quality of life. Quality of life can be measured with a standardized questionnaire, such as the National Eye Institute Refractive Error Quality of Life Instrument. Patients with better quality of life pre-operatively will benefit less from cataract surgery. This study aimed to describe patients' quality of life after long-term cataract surgery in the National Eye Center Cicendo Eye Hospital. This cross-sectional descriptive study used primary data collected using a questionnaire from March to May 2022. The subjects were 33 people with cataracts who underwent uncomplicated cataract surgery at Cicendo National Eye Hospital over the past three and six months of the study. Patients who met the inclusion criteria were given the NEI-RQL 42 questionnaire. In visual clarity, patient expectations, near vision, far vision, daily fluctuations, activity limitations, suboptimal correction, and satisfaction with correction variables, an increase in value at three months was observed, yet slightly decreased at 6 months. There was decreased glare complaints, symptoms, and dependence on correction at preoperative, 3 months, and 6 months. An increase in the level of concern and appearance from preoperative, 3 months, and 6 months post-operative was observed. Thus, an improvement in the patient's quality of life is observed three months after surgery, yet decreases slightly in six months after surgery.

Keywords: Cataract surgery, NEI-RQL 42, quality of life

Introduction

Cataracts are the main cause of reversible visual impairment in older populations. Cataract Prevalence rate in Indonesia was 23.0% and increased mainly in rural areas. Indonesia's countrywide prevalence of blindness was 3.0%, with untreated cataracts becoming the most common cause at 71%. Cataracts will affect a person's quality of life both physically, mentally, and socially. [1–5]

Cataract surgery consists of cataract removal and insertion of intraocular lens. There are several available techniques for cataract surgery. Phacoemulsification surgery is considered a cost-effective operation for cataracts that will improve a patient's visual acuity and quality of life. Cataract surgery can be performed unilaterally or bilaterally. However, surgery that is only performed on one eye will affect binocular abilities, hand coordination, and uncomfortable glasses.[1,4-8]

Several researchers have questioned the appropriateness and potential overuse of cataract surgery. A study conducted in the UK by Malik et al.,[9] found that 33% of patients undergoing cataract surgery had a visual acuity of 6/12 or better. However, visual impairment caused by cataracts cannot be fully captured by visual acuity alone. Patient symptoms and satisfaction are crucial for assessing their quality of life. Quality of life can be influenced by demographic factors such as gender, age, education, and occupation. Additionally, the type of surgery performed can lead to varying rates of aberration and astigmatism postoperatively. In the absence of formal assessments, questionnaires can be valuable in evaluating the impact of cataract surgery on patient quality of life. Some studies suggest that patients with minimal visual impairment may benefit less from cataract surgery, highlighting that visual acuity alone is

Corresponding Author:
Pieter Juanarta
Department of Ophthalmology, Faculty of Medicine
Universitas Padjadjaran/Cicendo National Eye Hospital,
Bandung, Indonesia
Email: pieter.juanarta@yahoo.com

not an ideal measure of overall visual function.[9–12] This study aims to evaluate the quality of life of patients following long-term cataract surgery at the National Eye Center, Cicendo Eye Hospital, during the period from March to May 2022. The data collection was conducted three months after the surgery, considering the high volume of uncomplicated cataract surgeries performed between December 2021 and February 2022.

Methods

This cross-sectional descriptive study utilizes both primary data collected through a questionnaire and secondary data from patient medical records. The study has received ethical approval from the National Eye Center Cicendo Eye Hospital's Ethics Committee (approval number LB.02.01/2.3/1988/2022). The study population included cataract patients who underwent cataract surgery at the National Eye Center Cicendo Eye Hospital between three and six months prior and were reachable during the data collection period from March to May 2022. The surgeries included phacoemulsification and small incision cataract surgery (SICS). Quality of life and visual function were assessed using the NEI-RQL 42 questionnaire, which is widely used for measuring these aspects in patients with visual acuity disorders. The NEI-RQL 42 comprises 42 questions divided into 13 categories, with each category containing one to seven questions. Higher scores on this questionnaire indicate a better quality of life. The questionnaire demonstrates excellent test-retest reliability (intraclass correlation coefficient [ICC], 0.91; 95% limits of agreement, -9.1 to 10.1) and good concurrent validity. The Indonesian version of the questionnaire was adapted using a forward-backward translation method. Scores obtained three- and six-months post-surgery provide insights into the patient's visual acuity quality following the receipt of appropriate corrective lenses.

The inclusion criteria for this study were adult patients aged 18 years and older who had undergone cataract surgery in both eyes. Exclusion criteria included patients who could not be contacted during the study period, those unable to complete the questionnaire, individuals with surgical complications, patients with incomplete medical records, those with a history of eye surgeries other than cataract surgery, patients who had cataract surgery combined with other surgical procedures, and individuals with other ocular disorders that could affect visual acuity.

Patients who meet the criteria will be contacted, come to the hospital, sign the informed consent, and be asked to fill out the NEI-RQL 42 questionnaire. Patients with symptoms are asked to return to the National Eye Center, Cicendo Eye Hospital. The selection of patients was carried out using the total sampling method.

The data taken consisted of the patient's demographic characteristics, gender, age, last education, and occupation. The level of patient education is classified based on the international standard education classification into Elementary School, Junior High School, Senior High School, and Higher Education. The patient's clinical characteristics consisted of the type of surgery, the intra-ocular lens used, the visual acuity before surgery, three months after surgery for the 3-month group, and 6 months after surgery for the 6-month group. Visual Acuity was measured using the Snellen chart and was categorized into no visual impairment if better seeing eye visual acuity was better than 6/12, mild visual impairment if worse than 6/12, moderate visual impairment if worse than 6/18, severe visual impairment if worse than 6/60, and blindness if worse than 3/60. Questionnaire data was taken based on the results of patient filling for conditions before surgery, three and six months after surgery. The data obtained will be processed and grouped using Microsoft Excel 2019. The results are presented in the table.[13,14]

Results

The total population in this study consists mainly of women (63.6%), with an average age of 58.18 years. The study population mostly only finished elementary school (54.6%) and worked as housewives (60.6%). Table 1 shows the demographic characteristics of the study.
In this study, patients mostly underwent phacoemulsification surgery in 55 eyes (83.3%) using 3-piece hydrophobic foldable acrylic lenses (33.3%) and 1-piece hydrophilic foldable acrylic lenses (33.3%). At preoperative, 44 eyes (66.7%) were blind, and postoperatively, 54 eyes had no visual acuity disorder (81.8%).

Table 3 shows the percentage of questionnaire scores in patients before and after surgery for three and six months. In the level of visual clarity, patient expectations, near vision, far vision, diurnal fluctuations, activity limitations, suboptimal correction, and satisfaction with

Table 1 Demographic Characteristics of the Study

Variable	Total (n=33)
Age	58.18
Gender	
Male	12 (36.4%)
Female	21 (63.6%)
Education	
Elementary School	18 (54.6%)
Junior High School	2 (6.1%)
Senior High School	10 (30.3%)
Higher Education	3 (9.1%)
Occupation	
Housewives	20 (60.6%)
Retired	7 (21.2%)
Office Worker	5 (15.2%)
Driver	1 (3.0%)

correction, there was an increase in the 3-month after surgery but slightly decreased in the 6-month after surgery. There was a decrease in the variables of glare complaints, symptoms, and dependence on correction from preoperative, 3 months, and 6 months after surgery. There was an increase in the level of concern and appearance variables from preoperative, 3 months control, and 6 months post-operative.

Discussion

In this study, patient quality of life improved after cataract surgery compared to preoperative levels; however, there was a notable decline in scores from three to six months postoperatively. This decrease may be attributed to several factors, including posterior lens opacities, reduced corneal endothelial cell density, and post-surgical macular edema. These issues can impact patient expectations, as individuals who experienced clearer vision three months postoperatively might notice a decline in visual

Table 2 Clinical Characteristics of the Study

Variable	Total (n=66)
Operation	
Phacoemulsification	55 (83.3%)
SICS	11 (16.7%)
IOL	
3-piece hydrophobic foldable acrylic lenses	22 (33.3%)
1- piece hydrophilic foldable acrylic lenses	22 (33.3%)
1-piece hydrophobic acrylic lenses	14 (21.2%)
1-piece polymethyl methacrylate lenses	8 (12.1%)
Visual Impairment Pre-Operative	
No Visual Impairment	0 (0%)
Mild Visual Impairment	0 (0%)
Moderate Visual Impairment	14 (21.2%)
Severe Visual Impairment	8 (12.1%)
Blindness	44 (66.7%)
Visual Impairment Post-Operative	
No Visual Impairment	54 (81.8%)
Mild Visual Impairment	6 (9.1%)
Moderate Visual Impairment	6 (9.1%)
Severe Visual Impairment	0 (0%)
Blindness	0 (0%)

Table 3 Questionnaire Score 3 Months and 6 Months Post-Operative

Variable	Preoperative	3-Months After Surgery	6-Months After Surgery
	n=33	n=33	n=33
Visual Clarity	51.8	82.8	77.4
Patient Expectations	29.5	84.1	66.7
Near Vision	52.0	88.4	81.5
Far Vision	71.5	91.0	87.3
Diurnal Fluctuations	55.8	90.5	84.7
Activity Limitations	73.5	93.4	93.0
Glare	81.1	65.9	64.8
Symptoms	91.0	75.4	74.5
Dependence on Correction	72.3	32.7	32.3
Concern	55.3	81.4	82.4
Suboptimal Correction	53.0	74.6	69.3
Appearance	55.8	84.3	84.6
Satisfaction with Correction	39.4	78.2	75.2

function over time. Additionally, the most frequently reported complaint was dry eyes, which is a common symptom following cataract surgery and tends to resolve or improve within six months.[14-17]

It was found that the number of females was more than that of males; this is by previous studies, which said that the incidence of cataracts was higher in older females. A five-year survey of Britta et al.,[2] showed that long-term visual acuity after cataract surgery was worse in females than in males. Male also often require earlier surgery due to their need for better eyesight at work. In addition, research from Makabe et al[3] showed higher male scores on several specific variables such as driving which were thought to be related to higher driving experience. Previous studies have shown that females more often have visual complaints than males. This can be seen in the glare and symptom variables, which have quite low scores after surgery.[14-18]

Patient education level is related to patient quality of life, especially in reading ability. Patients with low levels of education often rarely read, so an increase in near reading ability does not increase patient quality of life. In addition, patients with a high level of education usually undergo surgery with milder visual acuity impairment than patients with a lower level of education. Limitations of patients in doing an activity affect patients' quality of life after surgery. Patients who work as housewives or retirees often do not feel any limitations in their daily activities, in contrast to patients who work as employees or drivers. This also affects the level of visual acuity before surgery in men who work, which is usually higher than those who do not. In this study, there was a higher value on the activity limitation variable, and it was maintained at six months postoperative control.[5,14,16,18,19]

Phacoemulsification is the choice of surgery in patients with immature cataracts, and SICS is more common in patients with mature cataracts. The phacoemulsification operation resulted in better uncorrected visual acuity than SICS, but the corrected visual acuity was not significantly different. This is due to the relatively high rate of astigmatism in patients with SICS, causing a high degree of glass dependence in these patients. One of the modifications of modern intraocular lenses is the aspheric shape of the design. This form of asphericity will reduce the level of aberrations and improve patients' visual acuity and quality of life. Lenses made of hydrophobic acrylic material and are three-part in shape have lower posterior capsule opacities and a more stable quality of life enhancement.[19-21]

The degree of visual impairment before and after surgery determines the level of the patient's quality of life. The quality of life will be higher in patients with a large difference between visual acuity before and after surgery. In addition, patients with poorer visual acuity will feel more significant improvement after surgery.[14,17,18]

The limitation of this study is that all questionnaires were filled out after cataract

surgery, which may introduce recall bias in the study population. Future research is needed to analyze factors affecting patients' quality of life after cataract surgery.

In conclusion, the majority of patients in this study were housewives, with an average age of 58 years and a basic level of education. Phacoemulsification surgery, employing 1- and 3-piece foldable hydrophilic and hydrophobic acrylic aspheric intraocular lenses, was the most commonly performed procedure. Prior to surgery, most patients experienced moderate visual impairment or blindness. Postoperatively, the majority of patients demonstrated no visual impairment. Quality of life assessments indicated an improvement three months after surgery compared to preoperative levels; however, a slight decline was observed six months postoperatively.

References

1. de Mendonça PT, de Mendonça LT, Rosa AAM, Silveira LC de L. Life quality assessment of patients after phacoemulsification or extracapsular cataract extraction. Arq Bras Oftalmol. 2014;77(1):12–6. doi:10.5935/0004-2749.20140005
2. Ka AM, Sow AS, Diagne JP, Ndoye Roth PA, Kamara K, De Medeiros ME, et al. [Patients' quality of life after cataract surgery]. J Fr Ophtalmol. 2017;40(8):629–35.
3. Jain S, Rajshekar K, Aggarwal A, Chauhan A, Gauba VK. Effects of cataract surgery and intra-ocular lens implantation on visual function and quality of life in age-related cataract patients: A systematic review protocol. Syst Rev. 2019;8(1):1–6. doi:10.1186/s13643-019-1113-6
4. Javed U, Mcveigh K, Scott NW, Azuara-Blanco A. Cataract extraction and patient vision-related quality of life: A cohort study. Eye. 2015;29(7):921–5. doi:10.1038/eye.2015.70
5. Kuntorini MW, Achmad MP, Halim A. Impact of First Eye Cataract Surgery on Quality of Life. MKB. 2023;55(1):27–34.
6. Lin IC, Wang IJ, Lei MS, Hu FR, Lin LL k. Refractive-error quality of life (RQL) in patients with AcrySof IQ aspherical intraocular lenses. J Optom. 2010;3(1):44–50.
7. Han J V., Patel D V., Squirrell D, McGhee CNJ. Cystoid macular oedema following cataract surgery: A review. Clin Experiment Ophthalmol. 2019;47(3):346–56.
8. McAlinden C, Skiadaresi E, Moore J, Pesudovs K. Subscale assessment of the NEI-RQL-42 questionnaire with rasch analysis. Investig Ophthalmol Vis Sci. 2011;52(8):5685–94. doi:10.1167/iovs.10-67951
9. Malik R, Frost NA, Sparrow JM, Hale L. The Bristol cataract listing survey: profile of listed patients with visual acuity 6/12 or better. Eye. 2005;19(4):406–10.
10. Pedrotti E, Carones F, Talli P, Bonacci E, Selvi F, Galzignato A, et al. Comparative analysis of objective and subjective outcomes of two different intraocular lenses: trifocal and extended range of vision. BMJ Open Ophthalmol. 2020;5(1):497–505. doi:10.1136/bmjophth-2020-000497
11. International Standard Classification of Education (ISCED) - ILOSTAT [Internet] 2022 [cited 2022 October 2]. Available from: https://ilostat.ilo.org/resources/concepts-and-definitions/classification-education/
12. Blancafort Alias S, Del Z, Carrasco C, Salvador-Miras I, Luna Mariné S, José Gómez Prieto M, et al. Exploring vision-related quality of life: a qualitative study comparing patients' experience of cataract surgery with a standard monofocal IOL and an enhanced monofocal IOL. Clin Ophthalmol. 2022;16:1641-1652. doi:10.2147/OPTH.S358386
13. Xue W, Zhu M ming, Zhu B jun, Huang J nan, Sun Q, Miao Y yu, et al. Long-term impact of dry eye symptoms on vision-related quality of life after phacoemulsification surgery. Int Ophthalmol. 2019;39(2):419–29. doi:10.1007/s10792-018-0828-z
14. Lundqvist B, Mönestam E. Gender-related differences in cataract surgery outcome: A 5-year follow-up. Acta Ophthalmol. 2008;86(5):543–8. doi:10.1111/j.1600-0420.2007.01099.x
15. Makabe K, Oshika T, Inamura M, Hayashi K, Sugita G, Kozawa T, et al. Influence of cataract surgery for the first or second eye on vision-related quality of life (VR-QOL) and the predictive factors of VR-QOL improvement. Japanese J Ophthalmo. 2020;64(5):468–77.
16. Zhu B, Ma Y, Lin S, Zou H. Vision-related quality of life and visual outcomes from cataract surgery in patients with vision-threatening diabetic retinopathy: A prospective observational study. Health Qual Life Outcomes. 2017;15(1):1–7.
17. Kyei S, Zaabaar E, Assiamah F, Kwarteng MA, Asiedu K. Comparison of the outcomes

of manual small incision cataract surgery (MSICS) and phacoemulsification (PHACO) in Ghana. Ann African Surg. 2021;18(3):143–9.
18. 18. Zhao Y, Yang K, Li J, Huang Y, Zhu S. Comparison of hydrophobic and hydrophilic intraocular lens in preventing posterior capsule opacification after cataract surgery An updated meta-analysis. Med (United States). 2017;96(44):1-9.
19. Yigit U, Cavusoglu E, Onur IU, Kaya FS. Clinical Outcomes Following Implantation of A New Aspheric Monofocal Intraocular Lens. J Acad Res Med. 2019;9(1):35–40.
20. Hussain S, Batool A, Akram S, Manzoor H, Arshad A. Comparison of post-op quality of vision of spheric IOL and aspheric hydrophilic acrylic yellow tinted intraocular lens implantation following phacoemulsification cataract surgery. Adv Ophthalmol Vis Syst. 2021;11(2):28–31.
21. Choi JY, Han YK. Long-term (≥10 years) results of corneal endothelial cell loss after cataract surgery. Can J Ophthalmol. 2019;54(4):438–44.

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Prostate Specific Antigen Level and Gleason Score in Indonesian Prostate Cancer Patients

Muhammad Imam Al Kautsar Herawan, Ricky Adriansjah
Department of Urology, Faculty of Medicine Universitas Padjadjaran
Dr. Hasan Sadikin General Hospital Bandung, Indonesia

Abstract

In Indonesia, the number of prostate cancer continues to increase and even becomes the most common malignancy in men in 2015. Delayed early detection of prostate cancer is a significant concern. Prostate-specific antigen (PSA) level is found to be elevated in various conditions of prostate tissue damage, including prostate cancer. The extent of tissue damage in prostate cancer, represented by the Gleason score, is suspected to be related to the increase in the PSA level in the bloodstream. To investigate the relationship between the PSA level and Gleason scores in prostate cancer patients, a cross-sectional observational study was conducted. The study population consisted of 83 prostate cancer patients treated in Dr. Hasan Sadikin General Hospital Bandung, Indonesia, from 2017 to 2021. Data collected were analyzed using Spearman correlation test was used with an alpha value of 5%. Results indicated that 37.3% patients had a PSA level above 200 ng/dL, and 25.3% had a PSA level between 10-50 ng/dL. The majority of patients (48.1%) had a Gleason score of 9-10 (ISUP 5), while 24.1% had a score of 8 (ISUP 4). Bivariate analysis showed no significant relationship between the PSA level and Gleason score (p-value=0.445). Further studies are needed to determine the sensitivity and specificity of PSA in diagnosing prostate cancer.

Keywords: Gleason grading, prostate cancer, prostate-specific antigen

Introduction

Prostate cancer is the 4th most common cancer globally and the 2nd most common cancer in men.[1] As of 2018, it is estimated that there will be 164,690 new cases of prostate cancer, with 29,430 deaths.[2] Reports of prostate cancer have increased in the Asian region over the past ten years, with an incidence rate of 4.1 per 100,000. In Indonesia, based on histopathological data from the IAPI Cancer Registration Agency and the Indonesian Ministry of Health in 2015, prostate cancer was ranked first as the most common tumor in men.[5] According to data from the Indonesian Society of Urologic Oncology (ISUO) in 2011, in the period 2006-2010 there were 971 prostate cancer patients with an average age of 68.3 years.[6] At Dr. Hasan Sadikin General Hospital in Bandung, 318 cases were reported between 2004 and 2010, with 193 cases (60.7%) being confined organs and 125 cases (39.3%) being metastatic.[6]

Prostate-specific antigen (PSA) is a serine protease enzyme produced by epithelial cells in the prostate gland. In prostate cancer, damage to the basal cell layer and basement cell membrane, along with increased angiogenesis, leads to PSA leakage into the bloodstream. PSA is organ-specific but not cancer-specific; an increase in PSA value can be caused by several conditions other than malignancy, such as urinary tract infections, prostatitis, BPH, or medical procedures such as the digital rectum and TURP.[11] In Indonesia, the PSA standard value used is 4 ng/ml.[7] When an increase of more than 4 ng/ml, serum PSA has a specificity of up to 91% in detecting prostate cancer.[8] At PSA levels > 4 ng/ml, prostate biopsy is highly recommended for the diagnosis and prognosis of prostate cancer.[9]

In determining the degree of malignancy of prostate cancer, the Gleason score is used as a parameter. Gleason's degree divides the pattern of histopathological findings of prostate cancer into 5 patterns.[10] Prostate cancer is known to have more than one Gleason pattern/degree; therefore, the Gleason Score is formed by

Corresponding Author:
Muhammad Imam Al Kautsar H.
Department of Urology, Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital Bandung, Indonesia
Email: imam.1303@yahoo.com

adding up the most dominant and second most dominant Gleason degrees and grouping them into groups. The Gleason score is also used as one assessment for determining prostate cancer staging (according to AJCC). It is also used as a predictive determination of prostate cancer (Partin Table) and therapeutic modalities.

Amarneel et al.[12] stated that there was a significant correlation between serum PSA and Gleason Histological Grade in patients with prostate cancer. This is also in line with the study of Jayapradeep et al.,[13] which states that high serum PSA levels are associated with the Gleason Score. However, a study by Gurumurthy et al.[14] stated no relationship between increased PSA levels and increased Gleason degrees.Research on the relationship between PSA levels and Gleason scores in prostate cancer has not been conducted at Dr. Hasan Sadikin General Hospital in Bandung, a leading referral hospital for West Java Province. This gap in research has prompted the author to undertake this study. The findings are anticipated to provide valuable data on prostate cancer patients and offer insights into diagnosing and assessing the severity of prostate cancer.

Methods

An observational cross-sectional study was conducted for this research. All men who had been diagnosed with prostate cancer and had prostate biopsies and histological tests between 2017 and 2021 made up the study population. Populations that matched the inclusion and exclusion criteria made up the whole sample for the research. Patients with prostate cancer who underwent PSA testing, histological analysis, and Gleason Score evaluation met the inclusion criteria. Patients with BPH, urinary tract infections, prostatitis, and insufficient medical record data were among those who were excluded. Eighty-three patients made up the study's final total sample.

PSA levels were categorized using an ordinal measurement scale based on intervals of 50 ng/dL. The Gleason score, determined by summing the Gleason grades and utilizing the International Society of Urological Pathology (ISUP) grouping system, was also assessed. Additionally, patient age and the number of years since treatment were considered in this study. Data were sourced from the medical records at Dr. Hasan Sadikin General Hospital in Bandung. The analysis employed the Spearman correlation test and descriptive statistics, with a significance level set at 5% (p-value<0.05), using SPSS version 23. Ethical approval for the study was granted by the Health Research Ethics Committee of Dr. Hasan Sadikin General Hospital under reference number LB.02.01/X.6.5/246/2023.

Results

Table 1 shows that the majority of patients were admitted in 2017 and 2018, accounting for 27.7% and 20.5% of the total cases, respectively. Regarding age, the predominant group consisted of patients older than 60 years. In terms of PSA levels, 37.3% of patients had levels exceeding 200 ng/dL, while 25.3% had PSA levels ranging from 10-50 ng/dL. Analysis of the Gleason scores revealed that most patients had scores in the 9-10 (ISUP 5) and 8 (ISUP 4) categories, representing 48.1% and 24.1% of the patient population, respectively.

The normality test of PSA serum level data and Gleason score in prostate cancer patients used the Komlogorov-Smirnov test, and it was found that the data distribution was not normal (p=0.000). The bivariate analysis between serum PSA levels and the Gleason score (Table 2) showed a p-value of 0.445; at 5% α, it can be concluded that there was no significant correlation between PSA levels and the Gleason score. In addition, an r correlation value of 0.085 was also obtained, which indicated a tendency for a weak correlation/no correlation between PSA serum levels and the Gleason score.

Discussion

Out of the 83 data points collected, an analysis of the distribution of prostate cancer patients at Dr. Hasan Sadikin General Hospital in Bandung from 2017 to 2021 was performed. The year 2017 recorded the highest number of prostate cancer patients, with 23 patients (27.7%). This was followed by 2019 with 20 patients (24%) and 2018 with 17 patients (20.5%). In contrast, the number of prostate cancer patients in 2020 and 2021 was relatively lower, with 14 patients (16.9%) in 2020 and 9 patients (10.8%) in 2021. The decrease in patient numbers in 2020 and 2021 is suspected to be linked to the COVID-19 pandemic in Indonesia, which led to a higher volume of COVID-19 patients visiting health services. This observation is consistent with a survey conducted by the WHO across 155

Table 1 Patient Characteristics

Variable		n
Year of admission	2017	23 (27.7)
	2018	17 (20.5)
	2019	20 (24)
	2020	14 (16.9)
	2021	9 (10.8)
Age group (years old)	45–50	3 (3.6)
	51–55	1 (1.2)
	56–60	9 (10.8)
	61–65	20 (24.1)
	66–70	20 (24.1)
	>70	30 (36.1)
PSA (ng/dl)	<10	10 (12)
	10–50	21 (25.3)
	51–100	14 (16.9)
	101–150	6 (7.2)
	151–200	1 (1.2)
	>200	31 (37.3)
Gleason score	≤ 6 (ISUP 1)	7 (8.4)
	7 (3+4) (ISUP 2)	3 (3.6)
	7 (4+3) (ISUP 3)	13 (15.7)
	8 (ISUP 4)	20 (24.1)
	9–10 (ISUP 5)	40 (48.1)

**PSA=prostate specific antigen; ISUP=International Society of Urological Pathology

countries, where 53% of respondents reported a decline in visits for non-communicable diseases, such as cardiovascular diseases, diabetes, and cancer, during the COVID-19 pandemic.[15]

The distribution of prostate cancer patients by age showed that the highest number was in the age group over 70 years with 30 patients (36.1%), followed by the age groups 61-65 years

Table 2 Bivariate Analysis between PSA Serum with Gleason Score

PSA serum (ng/mL)	Gleason Score										p-value	R Correlation
	≤6		7(3+4)		7(4+3)		8		9–10			
	n	%	n	%	n	%	n	%	n	%		
<10	3	3.6	0	0	1	1.2	3	3.6	3	3.6	0.445	0.085
10-50	2	2.4	0	0	3	3.6	6	7.2	10	12.0		
51-100	1	1.2	0	0	2	2.4	4	4.8	7	8.4		
101-150	1	1.2	0	0	2	2.4	1	1.2	2	2.4		
151-200	0	0	0	0	0	0	0	0	1	1.2		
>200	0	0	3	3.6	5	6.0	6	7.2	17	20.5		

Normality test result PSA: p-value 0.001; gleason score: p-value 0.001

and 66–70 years with 20 patients each (24.1%). This is in line with previous research conducted by Amarneel et al., which found that prostate cancer patients were more prevalent in patients over the age of 60, accounting 66.7% of total cases.[12] Similar results were also mentioned in a previous study conducted by Umbas, which indicated the incidence of prostate cancer would increase in the age group over 65 years and is very rare in those under 50 years of age.[16] The number of prostate cancer patients in the 56-60 age group was 9 patients (10.8%), 1 patient (1.2%) in the 51–55 year age group, and 3 patients (3.6%) in the 45–50 age group, with the youngest patient being 45 years old.

PSA is a serine protease produced by prostate epithelial cells, also known to be produced by prostate cancer cells. Some sources say that the ability of prostate cancer cells to secrete PSA is lower compared to normal prostate cells; however, PSA in prostate cancer cells penetrates the blood circulation more easily due to damage to the prostate basement membrane and its ability to avoid proteolysis in the circulation, thus increasing PSA serum levels in prostate cancer cases.[17]

This study found an overall increase in serum PSA levels among prostate cancer patients, with varying values. The largest proportion of patients had serum PSA levels exceeding 200 ng/ml, totaling 31 patients (37.3%). Conversely, the groups with lower PSA levels had fewer patients: 6 patients (7.2%) had PSA levels between 101–150 ng/mL, and only 1 patient (1.2%) was in the 151–200 ng/mL range. The second highest number of patients was in the PSA 10–50 ng/mL group, with 21 patients (25.3%). This was followed by the PSA 51-100 ng/mL group, which had 14 patients (16.9%), and the PSA <10 ng/mL group, which included 10 patients (12%).

In this study, it was found that the highest distribution of prostate cancer patients was at Gleason score 9–10 (ISUP degree 5) with 40 patients (48.1%), followed by the Gleason score group 8 (ISUP degree 4) with 20 patients (24.1%), 13 patients (15.7%) in the Gleason score group 7 (4+3) (ISUP Degree 3), while in the Gleason score group 6 (ISUP degree 1) there were 7 patients (8.4%), and the least was in the Gleason score group 7 (3+4) (ISUP degree 2) with 3 patients (3.6%). This is in line with the research conducted by Pai et al., which stated that many patients with adenocarcinoma had a higher histopathological degree of 77.77%.[18] Similar results were also mentioned in a study by Nina, which stated that a Gleason score of 2–4 was typically found as a small tumor in the transitional zone, often discovered accidentally when examining a TURP specimen with suspected BPH. Most cancers found in needle biopsy preparations have a Gleason score between 5 and 7.

A normality test was carried out on the PSA serum level data and the Gleason score was used using the Komlogorov-Smirnov test. The results obtained were p=0.000, indicating that the data distribution was abnormal. Consequently, data transformation was performed, and the result was p=0.000, confirming the abnormal distribution of the data. Therefore, data analysis in this study was conducted using a non-parametric test, specifically the Spearman correlation test with a 95% confidence level. The results of the Spearman correlation test obtained p=0.445 and r=0.085; since the p-value was >0.05, so it can be concluded that there was no significant correlation between serum PSA levels and the Gleason score in this study. Similar findings were also reported in a previous study conducted in India, which found no relationship between serum PSA levels and the Gleason score in prostate cancer patients, it was stated that 17.5% of study subjects with a Gleason score of 8-10 had low serum PSA levels. Some studies mention that prostate cancer cells produce more PSA than normal cells, thus poorly differentiated cancer cells release greater amounts of PSA than well-differentiated ones. However, some studies also mention that the lack of correlation between Gleason score and PSA may be explained by a decrease in antigen production by higher-grade lesions due to loss of expression of the PSA encoding gene.[14] A few weaknesses of this study are acknowledged: the small sample size and the lack of comprehensive medical records, which resulted in many samples being excluded.

In conclusion, from 2017 to 2021, 37.3% of prostate cancer patients at Dr. Hasan Sadikin General Hospital in Bandung had blood PSA levels exceeding 200 ng/mL, while 48.1% had Gleason scores of 9–10 (ISUP 5). During this period, there was no significant link between PSA serum levels and Gleason scores among prostate cancer patients at the hospital.

References

1. WHO. Cancer 2022. [Internet] 2022. Available from: https://www.who.int/news-room/fact-sheets/detail/cancer.
2. American Cancer Society. Key statistics

for prostate cancer: Prostate cancer facts. [Internet] 2023 [cited 2023 Mei 25]. Available from: https://www.cancer.org/cancer/types/prostate-cancer/about/key-statistics.html
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–49. doi:10.3322/caac.21660
4. Duffy MJ. Tumor markers in clinical practice: a review focusing on common solid cancers. Med Princ Pract. 2013;22(1):4–11. doi:10.1159/000338393
5. Safriadi F, Novesar AR. Five-Year profiles of prostate cancer patients in a tertiary Hospital in Indonesia. MKB. 2021;53(2): 101–6. doi:10.15395/mkb.v53n2.2212
6. Umbas R. Panduan penatalaksanaan kanker prostat. Jakarta: Ikatan Ahli Urologi Indonesia; 2022.
7. Laccetti AL, Morris MJ, Kantoff PW. A Clinical evaluation of enzalutamide in metastatic castration-sensitive prostate cancer: guiding principles for treatment selection and perspectives on research. Onco Targets Ther. 2020;13:13247–63. doi:10.2147/OTT.S242921
8. Albertsen PC. Prostate cancer screening with prostate-specific antigen: Where are we going?. Cancer. 2018;124(3):453–55. doi:10.1002/cncr.31140
9. Van Poppel H, Roobol MJ, Chapple CR, Catto JWF, N'Dow J, Sønksen J, et al. Prostate-specific antigen testing as part of a risk-adapted early detection strategy for prostate cancer: European Association of Urology position and recommendations for 2021. Eur Urol. 2021;80(6):703–11. doi:10.1016/j.eururo.2021.07.024
10. Chen N, Zhou Q. The evolving Gleason grading system. Chin J Cancer Res. 2016;28(1):58–64. doi:10.3978/j.issn.1000-9604.2016.02.04
11. Fang Y, Zhou X, Qiu J, Wen X, Wang D, Pang J, et al. Serum PSA level and gleason scores of needle biopsy specimens predict the postoperative pathologic grade of prostate cancer. Acta Medica Mediterr. 2013;29(2):279–83.
12. Amarneel S, Gohil MR, Parmar KN, Gamit S, Vala M, Shukla D. Study of serum prostate specific antigen level in prostate biopsy specimens. International Journal of Innovative Research and Development. [Internet] 2015 [cited 2023 Mei 15]. Available from: http://www.ijird_ojs/article/view/135677/0.
13. Jayapradeep D, Prakash V, Philose T, Pai M. Histomorphologic Ccorrelation of PSA levels in prostatic pathology. Natl J Lab Med. 2017;6(4):28–32. https://doi.org/10.7860/NJLM/2017/30113:2262
14. Gurumurthy D. Prostate carcinoma: correlation of histopathology with serum prostate specific antigen. Science Journal of Clinical Medicine 2015;4:1. https://doi.org/10.11648/j.sjcm.s.2015040401.11.
15. Bhowmick NA, Oft J, Dorff T, Pal S, Agarwal N, Figlin RA, et al. COVID-19 and androgen-targeted therapy for prostate cancer patients. Endocr Relat Cancer. 2020;27(9):R281–92. doi:10.1530/ERC-20-0165
16. Jairath NK, Dal Pra A, Vince R, Dess RT, Jackson WC, Tosoian JJ, et al. A systematic review of the evidence for the decipher genomic classifier in prostate cancer. Eur Urol. 2021;79(3):374–83. https://doi.org/10.1016/j.eururo.2020.11.021.
17. Stephan C, Ralla B, Jung K. Prostate-specific antigen and other serum and urine markers in prostate cancer. Biochimica et Biophysica Acta (BBA) - Reviews on Cancer 2014;1846:99–112. https://doi.org/10.1016/j.bbcan.2014.04.001.
18. Das J, Paul AK, Haque N, Dan KK, Saha S, Khan TI, et al. Correlation of prostate specific antigen, gleason score and bone metastases in bone scan in prostate cancer patient. SAS J Med. 2021;7(3):85–9. https://doi.org/10.36347/sasjm.2021.v07i03.005

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Comparison of Different Cycloplegic Refraction Regimens for Children

Irawati Irfani, Fany Gunawan, Karmelita Satari, Maya Sari, Primawita Oktarima, Sesy Caesarya, Feti Karfiati

Department of Ophthalmology, Faculty of Medicine Universitas Padjadjaran
Cicendo National Eye Hospital, Bandung, Indonesia

Abstract

In the process of a pediatric eye exam, cycloplegic refraction is a crucial step. Since no single cycloplegic drug is ideal, a combination regimen is employed. This study compares the ocular (refractive power and pupil diameter) and systemic (blood pressure and heart rate) effects of administering a combination of 1% cyclopentolate and 2.5% phenylephrine, with or without 1% tropicamide, to children with refractive errors. This study aimed to discover a more feasible regimen for children. This was a single-blind study, a randomized clinical trial conducted from November–December 2020 in children with mild to moderate refractive errors. Group A received 1% cyclopentolate, 2.5% phenylephrine, and 1% tropicamide (SFT), whereas group B received 1% cyclopentolate and 2.5% phenylephrine (SF). The outcomes were measured using an auto refractometer and IOL Master® 700. Before and 60 minutes after medication was administered, blood pressure and heart rate were measured. There were 54 participants (108 eyes) with an average age of 12.85±2.84 years. Although the SFT group's refractive power and pupil width were greater than those of the SF group, the differences were not statistically significant (p=0.271 and p=0.088). Heart rate (p=0.521) and blood pressure (systolic p=0.201; diastolic p=0.950) did not significantly differ between the two groups. Despite mydriasis manifested more rapidly in the SFT group, there was no discernible difference in the cycloplegic effects between those groups. SF might be a more feasible regimen for cycloplegic refraction in children with refractive errors.

Keywords: Cyclopentolate, cycloplegic, mydriasis, phenylephrine, tropicamide

Introduction

The second most common factor contributing to vision impairment and blindness worldwide is uncorrected refractive errors (URE). Up to 19 million children worldwide suffer from visual impairments, 12 million of which are brought on by URE. In Bandung's suburbs, children between the ages of 11 and 15 are more likely to have refractive errors (15.9%), with 12.1% of those cases remaining uncorrected.[1–4]

It's essential to address refractive errors in children since they can lead to blindness, amblyopia, as well as other visual impairments.[1,4] The high accommodation tone in children makes a cycloplegic examination a crucial part of a pediatric eye exam. The best cycloplegic medication should have minimal local or systemic side effects, a rapid onset of action, quick recovery, and adequate cycloplegia. The most common cycloplegic medications are cyclopentolate and tropicamide.[5–10] There are no recommendations on the best regimen for children, however many clinicians have moved to combination regimens and added sympathomimetic drugs, including phenylephrine, to enhance their mydriasis effect. When paired with tropicamide, cyclopentolate can enhance the cycloplegic effects, especially in eyes with dark irises. However, other investigations showed that tropicamide had a less potent effect on the same muscarinic receptors as cyclopentolate and did not change the latter's effect when it was withdrawn. While this is going on, using combinations of eye drugs can raise the chance of side effects, costs, and discomfort from stinging during instillation. The composition of the regimen, instillation patterns, and drug concentrations that are deemed best for children are not standardized.[5,8,10-12.]

The best treatment plans for children should minimize systemic and ocular side effects, reduce waiting times for examinations, lower

Corresponding Author:
Irawati Irfani
Department of Ophthalmology, Faculty of Medicine
Universitas Padjadjaran
Cicendo National Eye Hospital, Bandung, Indonesia
Email: irawati.irfani@unpad.ac.id

unnecessary operational costs, and alleviate discomfort for young patients. To address these concerns, we conducted a study to identify the most comfortable approach for pediatric patients. Specifically, if two drugs can achieve the same outcomes as three, we can decrease the number of drops administered, thereby enhancing comfort for children, reducing drug costs, and shortening clinic wait times. This study aims to compare the effects of 1% cyclopentolate and 2.5% phenylephrine, both with and without 1% tropicamide, on ocular parameters (changes in refractive power and pupil diameter) and systemic parameters (changes in blood pressure and heart rate) during cycloplegic refraction in children.

Methods

This single-blind, randomized clinical trial was conducted at the pediatric ophthalmology division of Cicendo National Eye Hospital in Bandung, Indonesia, from November to December 2020. The study adhered to the principles outlined in the Declaration of Helsinki and received approval from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Padjadjaran, under ethics number 985/UN6.KEP/EC/2020.

The trial included children aged 6 to 18 years who had mild to moderate refractive errors (myopia <6.00 D, hyperopia <3.00 D, and astigmatism <3.00 D) prior to the administration of cycloplegic agents. Exclusion criteria were as follows: history of eye surgery; pupillary abnormalities or other neurological disorders; history of cardiovascular disease; use of systemic or ocular medications affecting pupil function and/or accommodation; high anisometropia; visual disturbances unrelated to refractive errors (e.g., corneal opacities, uveitis, glaucoma, cataracts, posterior segment disorders, optic nerve disorders); strabismus; amblyopia; light-colored irises (e.g., green or blue); albinism; allergy to any component of the study drugs; and uncooperative behavior during drug administration. Additionally, children who failed to complete auto-refractometer and pupil diameter measurements or experienced adverse drug reactions were excluded from the study.

Block randomization was used to divide the sample into two groups. Group B received only 1% cyclopentolate and 2.5% phenylephrine (SF), while Group A received a combination of 1% cyclopentolate, 2.5% phenylephrine, and 1% tropicamide (SFT). Both eyes' visual acuity and autorefractometry were measured without the use of cycloplegics. The patient was then subject to an ophthalmological examination, which included assessing the patient's intraocular pressure, pupillary reflex, anterior segment, and funduscopy, as well as the position and movement of the patient's eyes. The study comprised patients who matched the inclusion criteria and did not meet the exclusion criteria.

The following dosage instructions were followed: (1) The SFT regimen consists of one drop each of 1% cyclopentolate, 2.5% phenylephrine, and 1% tropicamide; (2) The SF regimen consists of one drop each of 1% cyclopentolate and 2.5% phenylephrine. Each drop is administered in 5-minute intervals. One drop of 0.5% tetracaine is given 5 minutes before the first drop of the regimen. The lacrimal punctum is closed after each drop of the drug by applying pressure to the medial canthus for 10 seconds. IOL Master® 700 and an autorefractometer were used to measure the refractive power and pupil diameter prior to, 20, 30, 45, and 60 minutes following the administration of the medication. Each examination used the same intensity of room illumination. Before and 60 minutes after the administration of the medication, blood pressure and heart rate were measured.

The difference between refractive power before and after drug administration was the change in refractive power. For myopia and hyperopia, spherical power was applied in the calculation; for astigmatism, spherical equivalent (SE) was used. The difference between the pupil's size before and after the drug's delivery was the change in pupil diameter. The time started with the first drop of the regimen when the refractive power is achieved and maintained within 0.25 D of the previous refractive power is known as the cycloplegic peak time. The time measured from the first drop of the regimen with a pupil diameter greater than or equal to 7.0 mm is the mydriasis peak time. Peak periods of mydriasis and cycloplegia were recorded at 20, 30, 45, and 60 minutes.

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) formed up the blood pressure. Before and after the administration of medications, the value of the difference between SBP, DBP, and heart rate (HR) was measured. It expressed itself as a change in systolic blood pressure (ΔSBP), a change in diastolic blood pressure (ΔDBP), and a change in heart rate (ΔHR).

Data analysis was conducted using IBM SPSS

Statistics for Windows 26.0 and Microsoft® Excel 2010. Descriptive statistics were presented in tables and figures. To evaluate changes in refractive power and pupil diameter between the two groups, both the unpaired t-test and the Mann-Whitney test were employed. The peak times for cycloplegia and mydriasis were compared between the groups using the Chi-square test and the Kolmogorov-Smirnov test. Differences in diastolic blood pressure and heart rate between the groups were analyzed using the Mann-Whitney test, while systolic blood pressure was assessed with the unpaired t-test. A p-value of <0.05 was considered statistically significant.

Results

Table 1 shows the characteristics of the subjects in each group. There were 54 patients in total (108 eyes), with a median age of 13 years (mean 12.85±2.84 years). Forty girls (74.1%) and 14 boys (25.9%) composed the subjects. Astigmatism myopia compound (51.9%) and simple myopia (35.2%) were the most common refractive errors. All subjects had an average refractive power of -2.77±2.092 D with a pupil diameter of 5.61±0.70 mm.

Table 2 presents the changes in refractive power and pupil size following drug administration. At 20-, 30-, 45-, and 60-minutes post-administration, both parameters showed increasing trends in each group, indicating a reduction in accommodation and progressive pupil dilation. Throughout these time points, the mean values of refractive power and pupil diameter were higher in the SFT group compared to the SF group. However, there were no significant differences between the two groups regarding changes in refractive power. Pupil diameter did not show significant

Table 1 Comparison of the Subject Characteristics Between SFT and SF

	SFT (n=54)	SF (n=54)	p-value
Sex			0.667[a]
Boys	14 (25.9%)	16 (29.6%)	
Girls	40 (74.1%)	38 (70.4%)	
Age (years)			0.921[b]
Mean ± SD	12.85 ± 2.929	12.85 ± 2.771	
Age category			1.000[a]
6–12 years	20 (37.0%)	20 (37.0%)	
13–18 years	34 (63.0%)	34 (63.0%)	
Refractive state			1.000[c]
Simple myopia	19 (35.2%)	16 (29.6%)	
Simple Hypermetropia	0 (0.00%)	2 (3.7%)	
Astigmatism Myopia Simple	2 (3.7%)	6 (11.1%)	
Astigmatism Myopia Compound	28 (51.9%)	27 (50.0%)	
Astigmatism Hypermetrop Simple	3 (5.6%)	0 (0.00%)	
Astigmatism Hypermetrop Compound	1 (1.9%)	0 (0.00%)	
Astigmatism Mixtus	1 (1.9%)	3 (5.6%)	
Refractive power(D)			0.220[b]
Mean±SD	-2.98 ± 2.024	-2.56 ± 2.156	
Pupil diameter (mm)			0.946[d]
Mean±SD	5.61 ± 0.673	5.60 ± 0.733	

Notes: SFT: combination of 1% cyclopentolate, 2.5% phenylephrine, and 1% tropicamide; SF: combination of 1% cyclopentolate and 2.5% phenylephrine; [a]Chi-square test; [b]Mann-Whitney test; [c]Kolmogorov-Smirnov test; [d]Unpaired t test

Table 2. Comparison of the Differences Between SFT and SF's Refractive Power, Pupil Diameter, Cycloplegic and Mydriasis Peak Time, Blood Pressure and Heart Rate Variation

		SFT (n=54)	SF (n=54)	p-value
ΔRefractive power (D) (Mean ± SD)				
20 minutes		+0.36 ± 0.499	+0.25 ± 0.375	0.357[a]
30 minutes		+0.43 ± 0.542	+0.27 ± 0.401	0.104[a]
45 minutes		+0.46 ± 0.542	+0.36 ± 0.428	0.382[a]
60 minutes		+0.50 ± 0.551	+0.37 ± 0.428	0.271[a]
ΔPupil diameter (mm) (Mean ± SD)				
20 minutes		1.26 ± 0.721	1.01 ± 0.615	0.089[a]
30 minutes		2.36 ± 0.805	1.64 ± 0.709	0.0001*[d]
45 minutes		2.85 ± 0.841	2.38 ± 0.761	0.003*[b]
60 minutes		3.04 ± 0.878	2.77 ± 0.734	0.088[b]
Cycloplegic peak time				1.000[c]
Unable to identified[a']		29 (53.7%)	32 (59.3%)	
20 minutes		19 (35.2%)	14 (25.9%)	
30 minutes		2 (3.7%)	3 (5.6%)	
45 minutes		2 (3.7%)	5 (9.3%)	
60 minutes		2 (3.7%)	0 (0.0%)	
Mydriasis peak time				0.031[c*]
20 minutes		27 (50.0%)	18 (33.3%)	
30 minutes		25 (46.3%)	19 (35.2%)	
45 minutes		2 (3.7%)	15 (27.8%)	
60 minutes		0 (0.0%)	2 (3.7%)	
ΔSBP (mmHg)[a]	Mean ± SD Range	2.37 ± 7.256 -12 – 18	0.30 ± 4.886 -10 – 10	0.201[b]
ΔDBP (mmHg)[a]	Mean ± SD Range	1.47 ± 6.361 -10 – 14	1.57 ± 5.987 -9 – 14	0.950[b]
ΔHR (times/minute)[a]	Mean ± SD Range	1.00 ± 4.386 -10 – 11	1.40 ± 4.407 -7 – 8	0.521[a]

Notes: SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate [a]Mann-Whitney test; [b]Unpaired t test; a':The difference in SE between before and 60 minutes after instillation was only ±0.25 D so that cycloplegic peak time could not be identified; [c]Kolmogorov-Smirnov test; Δ[a]: The value of the difference in measurement between before and after drug administration; *p value <0.05 is considered to have a statistically significant difference

differences between the groups at 20 and 60 minutes (p ≥ 0.05), although significant variation was observed at 30 and 45 minutes (< 0.05).

Due to the minimal difference in spherical equivalent (SE) refractive power (±0.25D), the cycloplegic peak time could not be determined for 32 subjects (59.3%) in the SF group and 29 subjects (53.7%) in the SFT group. The cycloplegic peak time did not show a statistically significant difference between the SFT and SF groups (p=0.031). Table 2 presents the peak times for cycloplegia and mydriasis in both groups. Peak times were reported as cumulative percentages, and data that could not be classified were excluded from the analysis. At 45 minutes, 92% of the SFT group and 100% of the SF group had reached their cycloplegic peak. In contrast, by the same time point, only 69% of the SF group had reached the mydriasis peak, while a total of 96% of the SFT group had achieved this

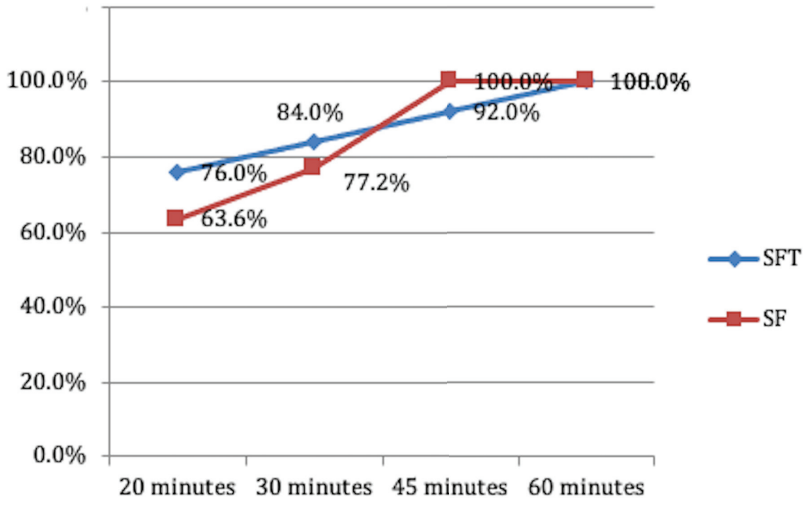

Figure 1.a Cumulative Percentage of Cycloplegic Peak Time Between SFT and SF

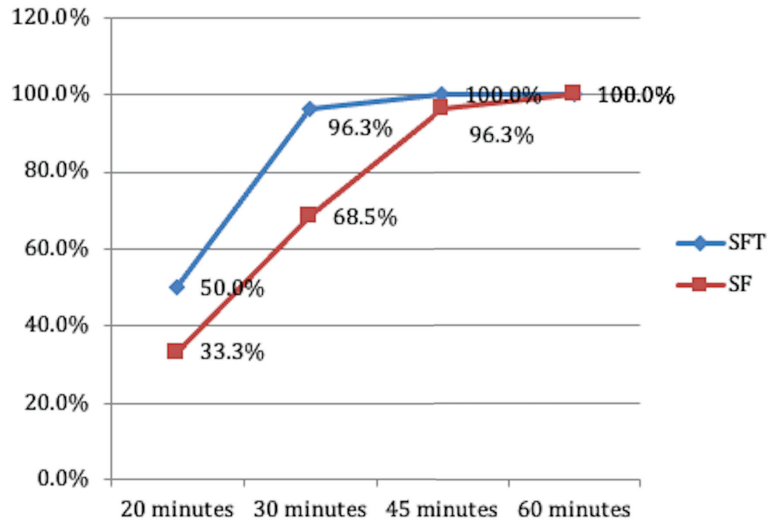

Figure 1.b Cumulative Percentage of Mydriasis Peak Time Between SFT and SF

milestone at 30 minutes.

This study compared the changes in blood pressure and heart rate to evaluate the systemic effects of various regimens in addition to their ocular effect. Table 2 shows that after drug administration in the SFT and SF regimens, the mean values of SBP, DBP, and HR increased. Systolic and diastolic blood pressure, as well as heart rate, were similar in both groups (p = 0.201; p=0.950) (p=0.521). However, as the systemic effect was only examined in this study at the 60th minute, fluctuations and variability could not be determined.

Figures 1.a and 1.b show the cumulative percentages of the cycloplegic and mydriasis peak times between two groups.

Discussion

This study demonstrated no significant difference in refractive power between the SFT and SF instillation methods. Cyclopentolate primarily acts on M1 muscarinic receptors,

while tropicamide affects M1, M2, and M4 receptors. When used together, tropicamide may enhance cyclopentolate's effect by suppressing M4 and M2 receptors, potentially leading to a more pronounced antimuscarinic effect overall. Despite these pharmacological interactions, the mean change in refractive power with SFT (0.13 D) was greater than with SF, but this difference was not statistically significant.[5,7,13.]

Results of this study was similar to that of Sherman[5] et al., which demonstrated that changes in refractive power caused by SFT and SF were not significantly different in the dark and light iris. Yoo et al.[9] compared a regimen consisting of 0.5% tropicamide, 0.5% phenylephrine combined with 1% cyclopentolate versus 1% cyclopentolate alone. The study showed that among hyperopic children aged 6 to 14 years, the two regimens did not significantly differ in their ability to produce cycloplegic symptoms. However, a cyclopentolate regimen alone produced a larger cycloplegic impact in children aged 5 and under than a combination of cyclopentolate with tropicamide and phenylephrine did, particularly in those with severe hyperopia or accommodative esotropia.[5,9] This study also shown that, despite a difference between SFT and SF during 30 and 45 minutes of observation, there was no discernible difference in the mean change in pupil diameter between the two groups. The SFT regimen produced pupils that were 0.72 mm and 0.47 mm larger than SF at 30 minutes and 45 minutes, respectively. When combined with cyclopentolate and phenylephrine, tropicamide has a quicker mydriasis effect because it penetrates the corneal epithelium more effectively than cyclopentolate.[13] Sherman[5] et al. demonstrated that SFT produced a pupil diameter that was 0.39 mm greater than SF in patients with dark irises, however the measurement was only made once, i.e., 30 minutes after installation.[5]

The peak timings of several cycloplegic drugs have also been investigated in several prior studies. Laojaroenwanit[6] et al. assessed the cycloplegic peak time of 3 drops of 1% cyclopentolate in Thai children and found that it took longer in participants with myopia—up to an hour—to reach the peak time (50 minutes). These studies were consistent to our findings, which demonstrated that most myopic participants had cycloplegic peak times in SFT and ST of 45 minutes.[6]

Contrary to this outcome, mydriasis peak time varied considerably between the two regimens. The SFT regimen induced mydriasis more rapidly than SF. Pupil diameter varied significantly during 30 and 45 minutes of observation. The mydriasis peak time was identified using 7.0 mm as the upper limit value because it is the optimum diameter for funduscopic examination.

Sherman et al.[5] investigated the effects of mydriasis in patients aged 4 to 32 years with dark irises, comparing two regimens: tropicamide 1% combined with phenylephrine 2.5% (TF) and tropicamide 1% combined with cyclopentolate 1% (TS). The study found that the TF regimen achieved a pupil diameter of 7 mm in an average of 32 minutes, while the TS regimen took an average of 52 minutes. Additionally, the study noted that younger patients required a longer time to reach the desired pupil diameter compared to older age groups.

This study also evaluated the safety of using the two combination regimens by comparing the likelihood of systemic effects on blood pressure and heart rate. The results showed no difference in heart rates or systolic and diastolic blood pressure between the two groups. The risk of adverse effects and costs can rise when using a range of medications. The higher volume of drops, stinging and burning sensations could induce trauma in children. Children become uneasy and less complaint throughout subsequent assessments. Additionally, cycloplegic refraction is carried out once the pupil has fully dilated and stopped responding to light. Because the maximum cycloplegic and mydriatic effects are uncertain, this prolongs waiting times. [5,12,17]

Based on the findings, the inclusion of tropicamide in the SFT regimen for pediatric cycloplegic refraction may not be necessary. Although tropicamide accelerates mydriasis, it does not significantly enhance the cycloplegic effect of the SFT regimen. Using only 2.5% phenylephrine can achieve adequate mydriasis without the additional systemic risks associated with higher concentrations of phenylephrine, such as tachycardia and elevated blood pressure, which is particularly relevant for infants and the elderly.[14]

The outcomes of this study suggest a more comfortable approach for cycloplegic evaluation in children. The effectiveness of two-drug regimens in inducing cycloplegia is comparable to that of three-drug regimens. As a result, using a two-drug regimen can reduce the discomfort associated with cycloplegic refractive examinations in children.

A limitation of this study is its focus primarily on myopic eyes, which may not fully represent the efficacy of the regimen for other refractive abnormalities. Further research is needed to

assess the benefits of this regimen for children with extreme hyperopia and accommodative esotropia. Additionally, exploring the effectiveness of drug combinations when synthesized into a single drop could provide further insights into optimizing cycloplegic treatments.

In conclusion, while mydriasis develops more rapidly with the SFT regimen, there is no significant difference in cycloplegic effects between the two groups. Both treatment regimens are systemically safe for children. The SF regimen may be a more practical option for cycloplegic refraction in children with refractive errors.

References

1. Dhaneshа U, Polack S, Bastawrous A, Banks LM. Prevalence and causes of visual impairment among schoolchildren in Mekelle, Ethiopia. Cogent Med. 2018;5:1554832.
2. Harrington SC, Stack J, Saunders K, O'Dwyer V. Refractive error and visual impairment in Ireland schoolchildren. Br J Ophthalmol. 2019;103(8):1112–8. doi:10.1136/bjophthalmol-2018-312573
3. WHO. World Report on Vision. Geneva: World Health Organization; 2019.
4. Halim A, Suganda R, Sirait NS, Memed FK, Syumarti, Rini M, et al. Prevalence and associated factors of uncorrected refractive errors among school children in suburban areas in Bandung, Indonesia. Cogent Med. 2020;7(1):1737354.
5. Sherman AE, Shaw MM, Ralay-Ranaivo H, Rahmani B. Tropicamide has limited clinical effect on cycloplegia and mydriasis when combined with cyclopentolate and phenylephrine. J AAPOS. 2019:2.e1-30.e5.
6. Laojaroenwanit S, Layanun V, Praneeprachachon P, Pukrushpan P. Time of maximum cycloplegia after instillation of cyclopentolate 1% in children with brown irises. Clin Ophthalmol. 2016;10:897–902.
7. Kyei S, Nketsiah AA, Asiedu K, Awuah A, Owusu-Ansah A. Onset and duration of cycloplegic action of 1% cyclopentolate – 1% tropicamide combination. Afr Health Sci. 2017;17(3):923–32. doi:10.4314/ahs.v17i3.36
8. Yazdani N, Sadeghi R, Momeni-Moghaddam H, Zarifmahmoudi L, Ehsaei A. Comparison of cyclopentolate versus tropicamide cycloplegia: A systematic review and meta-analysis. J Optom. 2018;11:135–43.
9. Yoo SG, Cho MJ, Kim US, Baek S-H. Cycloplegic refraction in hyperopic children: Effectiveness of a 0.5% tropicamide and 0.5% phenylephrine addition to 1% cyclopentolate regimen. Korean J Ophthalmol. 2017;31(3):249.
10. American Academy of Ophthalmology. Pediatric Eye Evaluations Preferred Practice Pattern®. San Francisco; Elsevier Inc.; 2017.
11. Minderhout HM, Joosse M V, Grootendorst DC, Schalij-Delfos NE. A randomized clinical trial using atropine, cyclopentolate, and tropicamide to compare refractive outcome in hypermetropic children with a dark iris; skin pigmentation and crying as significant factors for hypermetropic outcome. Strabismus. 2019;27(3):127–38. doi:10.1080/09273972.2019.1629466
12. Liewellyn S, Khandelwal P, Glaze S, Thomas P, Dahlmann-Noor A. Time to drop the phenylephrine from the paediatric cycloplegia protocol: informing practice through audit. Eye. 2019;33:337–8. doi:10.1038/s41433-018-0246-5
13. Gupta SK. Mydriatics and cycloplegics. In: Gupta SK, Agarwal R, Srivastava S, eds. Textbook on Clinical Ocular Pharmacology and Therapeutics. 1st ed. New Delhi: Jaypee Brothers Medical Publishers; 2014. p. 87–95.
14. Esteve-Taboada JJ, Del Aguila-Carrasco AJ, Bernal-Molina P, Ferrer-Blasco T, Lopez-Gil N, Montes-Mico R. Effect of phenylephrine on the accommodative system. J Ophthalmol. 2016;2016:1–13. doi:10.1155/2016/7968918

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Management of Stage-4 HIV with Cerebral Toxoplasmosis Coinfection and SIADH Complication

Pandu Laksono,[1] Erlin Oktavia,[1] Hidayati Adi Putri,[1] Fauna Herawati[2]
[1]Student of Master of Pharmaceutical Science Program, Faculty of Pharmacy, University of Surabaya, Surabaya, Indonesia
[2]Departement of Clinical and Community Pharmacy, Faculty of Pharmacy, University of Surabaya, Surabaya, Indonesia

Abstract

HIV/AIDS stage 4 is the stage where the HIV/AIDS patients have low immunity protection against infections, which can led to coinfections and complications. This case report presented an evaluation of the diagnosis and treatment of an HIV/AIDS stage 4 patient with cerebral toxoplasmosis coinfection and SIADH complications. A man (47 years, 35 kg), married with two children, complaining of weakness, nausea, vomiting, weight loss, and low appetite. Sodium level were measured, showing a 117 mg/dL level that continued to decrease throughout the patient's treatment. Five days later, the patient lost his consciousness with a GCS score of 224, indicating severe brain injury, and was diagnosed with cerebral toxoplasmosis based on the result of head CT-SCAN with contrast. On day 6, the patient was tested positive for HIV and diagnosed with stage 4 with an absolute CD4 count of 4 cells/µl. Therapy was provided by giving pyrimethamine-clindamycin therapy for cerebral toxoplasmosis, followed by Tenofovir, Lamivudine, and Evafirenz as antiretroviral therapy. Treatment for hyponatremia was done by administration of 3% NaCl and tolvaptan. The patient started experiencing an improvement in consciousness after the 10th day of medication, and sodium levels fluctuated throughout the treatments. Patient was discharged after 15 days with clinical improvements.

Keywords: HIV, acquired immunodeficiency syndrome, cerebral toxoplasmosis, inappropriate ADH syndrome, anti-retroviral agents, antiparasitic agents, hypertonic sodium chloride solution

Introduction

Human Immunodeficiency Virus (HIV) is a virus that infects white blood cells and causes a decrease in the human immune system, the infection causes a set of symptoms that can be known as Acquired Immune Deficiency Syndrome (AIDS).[1] There were 38.4 million cases with 650,000 deaths prevalence of HIV in the world based on WHO and the United Nations Programme on HIV/AIDS (UNAIDS) in 2021. Whereas, in Indonesia, there were 543,100 million cases of HIV/AIDS reported in 2020 with 30,137 deaths. In 2021, the number of positive HIV cases was the lowest in the last four years with 36,902 cases reported.[1,2]

HIV causes sufferers to experience decreased immunity, so that it is easier to get infected with various other infectious diseases. One of the infection that commonly occurs as a coinfection in People Living with HIV (PLHIV) at an advanced stage is cerebral toxoplasmosis which is associated with low CD4 count.[3,4] Cerebral Toxoplasmosis is an infection occurring in the brain or central nervous system (CNS) caused by the protozoa Toxoplasma gondii. Toxoplasma gondii can cause serious illness in immunocompromised patients such as in PLHIV, the most cases, involvement of CNS damage can cause encephalitis as one of the causes of death in PLHIV patients.[5,6]

Toxoplasmic encephalitis occurs in the advanced stages of HIV due to the reactivation of latent tissue cysts after a primary infection causes focal lesions in the brains of HIV/AIDS patients. Hyponatremia is a common electrolyte abnormality among AIDS patients, hyponatremia can occur in 20–80% of patients hospitalized with HIV or AIDS.[7] A common comorbidity of hyponatremia in HIV-infected patients is

Corresponding Author:
Fauna Herawati
Department of Ophthalmology, Faculty of Medicine
Universitas Padjadjaran
Cicendo National Eye Hospital, Bandung, Indonesia
Email: fauna@staff.ubaya.ac.id

coinfection of the lungs or CNS.[8] Coinfection that attacks both organs can cause excessive induction from the release of antidiuretic hormone (ADH) or also known as vasopressin, leading to Syndrome of Inappropriate Antidiuretic Hormone Secretion (SIADH). This case may explain why hyponatremia is inherent in this disease. In addition, hyponatremia also occurs due to adrenal insufficiency, renal impairment, and gastrointestinal sodium loss due to diarrhea and vomiting, which are observed in AIDS patients.[9,10,11] PLHIV, both with or without coinfection or complications, must receive antiretroviral therapy (ARV) aims to slow the progression of HIV that invades the patient's immune system. Especially, in conditions of coinfection with cerebral toxoplasmosis also requires pharmacological therapy and adequate sodium correction in the treatment of SIADH. It is necessary to monitor treatment closely because of the increased risk of unwanted events from polypharmacy, interactions between drugs, and even side effects of drugs that can overlap so that they interfere with or reduce the quality of treatment outcomes for PLHIV.[3,4,12]

This report will discuss a unique and rare case where stage 4 HIV disease has led to the development of cerebral toxoplasmosis co-infection accompanied by complicated SIADH. Previously, similar cases had been reported as a case series by Khosla et al., that discussed three patients who were treated with neuropsychiatric symptoms as well. These four cases were diagnosed with cerebral toxoplasmosis which later led to a diagnosis of HIV/AIDS.[13]

Comprehensive therapeutic management and collaboration between health professionals in the scope of infectious diseases and drugs are essential factors in achieving treatment success. Research by Urano et al. revealed that the role of pharmacists in HIV patient treatment programs in collaboration with doctors would improve the outcome of drug therapy. Based on the explanation above, this case report aims to report and explain in more detail the management and monitoring of drug therapy in the case of stage 4 HIV/AIDS patient with toxoplasmosis cerebral coinfection and complications of SIADH involving a collaboration of internal medicine specialists, neurologists, and pharmacists.[14]

Case

A man (35 kg, 160 cm), 47 years old, married, came to the hospital emergency room on April 2, 2021, complained of weakness since yesterday and getting worse, accompanied by nausea, vomiting, mouth ulcers, weight loss, and low appetite. Based on the anamnesis, the patient has a history of recurrent hyponatremia and taking salt capsules 3x500 g/day. The patient had no history of drug and food allergies or a family history of the disease. The results of an examination of the level of consciousness obtained a GCS score of 456 and vital signs such as blood pressure, pulse, temperature, respiratory rate, and oxygen saturation were within normal limits. On the same day, a laboratory examination was carried out in the form of a complete blood count that showed normal results, a serum electrolyte examination showed severe hyponatremia (117 mg/dL), a negative COVID-19 antigen swab, and a chest X-ray examination with readings showing no abnormalities. The patient was treated by the internist as the doctor in charge of the patient.

The next day, the correction for sodium level was done by administering an infusion of NaCl 3%/500 mL/24 hours 2 times. The patient experienced a decreased SpO2 <94%, so he mounted oxygen supplementation using an 8 liter per minute (lpm) oxygen mask. For each day, the patient experiences changes in the level of consciousness. On day 3, the patient's condition was unresponsive and decreased consciousness, accompanied by neck stiffness and hemiparesis, with a GCS score of 324. On April 6, 2021, the patient experienced diarrhea and an increase in body temperature of 37.8°C, a decrease in the GCS score of 224 with a total score of 8 indicates a severe injury to the brain. The CT-SCAN examination on the head was performed in contrast, and the results of its findings concluded that there was vasculitis and brain edema with a differential diagnosis of toxoplasmosis. The following is an overview of the imaging results:

Based on the clinical condition in the form of decreased consciousness and CT-scan results, the internist diagnosed cerebral toxoplasmosis and decided to consult this condition with neurologist. The neurologist suggested pyrimethamine 1x75 mg and clindamycin 4x600 mg as therapy for toxoplasmosis cerebral coinfection followed by the addition of 1x25 mg leucovorin oral therapy, 3x1 mg folic acid, and 1x62.5 mg methylprednisolone injection to reduce the edema in the brain. By the time of the therapy, there was an increase in patient awareness (GCS 446) on April 12, 2021.

During the hospital stay, the patient experienced a decrease in blood pressure, leading to the administration of norepinephrine

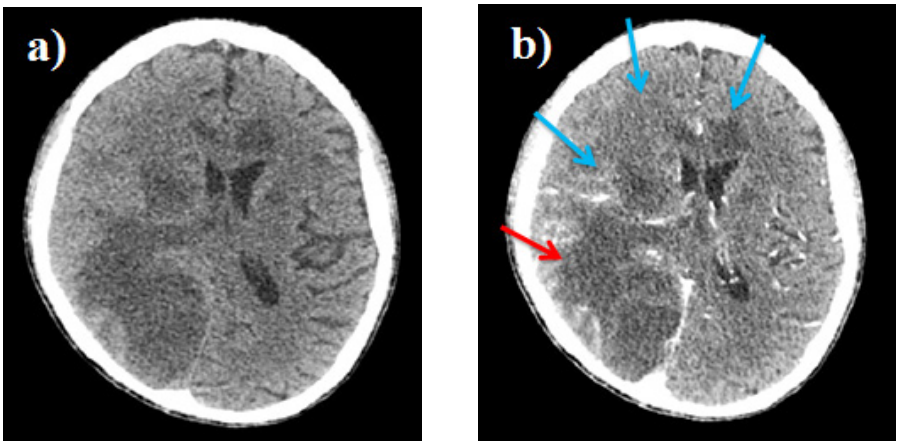

Figure 1 (a) CT-SCAN without contrast; (b) CT-SCAN with contrast

Edema Vaskulitis

as a vasopressor to target a blood pressure of 90/60 mmHg. On April 10, 2021, a follow-up laboratory examination revealed hemoglobin (Hb) levels of 8.3 g/dL and sodium at 123 mg/dL. To address these issues, the internist initiated treatment with NaCl 3% solution (500 mL over 24 hours) twice, and a transfusion of one unit of packed red cells per day to achieve a target Hb level of 10 g/dL. Additionally, the pyrimethamine dose was reduced to 25 mg once daily to mitigate

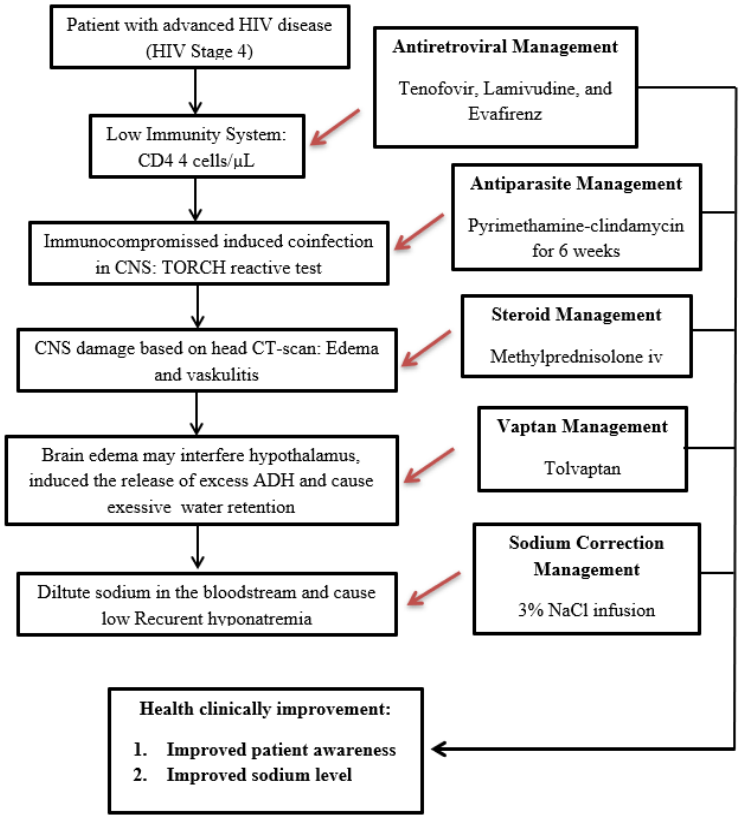

Figure 2 The Summary Flowchart of The Disease Progressivity and The Management of Each Clinical Issue

its side effects, specifically the reduction in folic acid contributing to low hemoglobin levels. By April 12, 2021, the patient's Glasgow Coma Scale (GCS) score improved to 456, indicating better communication and responsiveness.

The next day (April 13, 2021), post-transfusion laboratory examination of PRC and 3% NaCl in every 2 bottles was carried out with the result that the Hb target was reached (10.8 g/dL), while for natrium level it was still under the normal limit (121 mg/dL) by continuing 3% NaCl therapy until the 3rd unit. The neurologist recommended Tolvaptan therapy 1x15 mg to help correct sodium which has not improved. On April 16, 2021, the sodium electrolyte showed improvement (137 mg/dL), so the pharmacist suggested stopping tolvaptan therapy. On April 22, 2021, the patient's consciousness was stable and in good condition, able to communicate, answer questions, and move his hands and feet. The internist decided to start antiretroviral therapy (ARV), namely telavir with a composition of Tenofovir 300 mg, Lamivudin 300 mg, and Efavirenz 600 mg). On April 26, 2021, a liver function examination was carried out with the result of an increase of SGOT 86 U/L SGPT 205 U/L, so the patient was given a hepatoprotector Stronger Neo-Minophagen C (SNMC) injection one bottle/day). The increase in liver function was most likely caused by hepatotoxic drugs, in this case, efavirenz and cotrimoxazole, the decision to closely monitor together was given without stopping therapy which is highly suspected of being the cause of increased liver function in this case. On April 28, 2021, there was an improvement in liver function values SGOT 56 U/L SGPT 136 U/L and there were improvements in electrolytes Na 145 mg/dL. On May 2, 2021, liver function improved SGOT 44 U/L SGPT 72 U/L. The next day the patient's clinical condition improved, and the mouth ulcers and cough healed, GCS 456, as a result, the patient was allowed to be discharged with outpatient therapy. The summary of the disease progressivity and management of each clinical issue related to this case report is provided in Figure 2 in the appendix to navigate the complexities of treatment.

The study has received approval from the hospital management of Manyar Medical Centre, University of Surabaya, and adheres to Indonesian Law for the Protection of Personal Data as well as the Declaration of Helsinki. Written informed consent was obtained from the patient's family for participation in the study.

Discussion

A case of stage 4 HIV infection with coinfection with cerebral toxoplasmosis and complications of SIADH was found in a private hospital in Surabaya, Indonesia. In this case, starting from the results of the anamnesis, physical examination, and supporting examinations carried out, so that the diagnosis of opportunistic infections of HIV/AIDS could be established. This diagnosis was made based on the history of the symptoms of immunodeficiency in the form of diarrhea, yeast infection in the mouth, weight loss, nausea, vomiting, and fever. Furthermore, there was a history of same-sex sexual intercourse which could be a source of spread of HIV infection. On physical examination, the pain was found on abdominal pressure with BMI of 14.69 kg/m^2 which indicated malnutrition. Four days later, there was a decrease in the patient's level of consciousness with a total GCS score of 8 which signified a severe brain injury and followed by the results of a head CT-SCAN investigation with contrast. These results concluded that there were edema and lesions in the brain with a differential diagnosis of toxoplasmosis. The diagnosis was also supported by the results of a reactive TORCH examination with levels of 37.6 IU/mL, this confirmed the diagnosis of cerebral toxoplasmosis infection.[3,4,5,15]

According to WHO criteria, the patient in this case was diagnosed with stage 4 HIV as evidenced by a positive result on the 3-method HIV test with a CD4 count of 4 cells/μL and the presence of cerebral toxoplasmosis coinfection in the central nervous system. Cerebral toxoplasmosis is the most common cause of extensive brain lesions in PLHIV and leads to high morbidity and mortality. Especially, for PLHIV who did not receive cotrimoxazole and had a CD4 count <100 cells/mm^3, the results are illustrated as shown in this case. In immunosuppressed patients identical to this case, toxoplasmosis occurs as a result of reactivation of latent infection and causes neurological symptoms such as headache, disorientation, drowsiness, and hemiparesis (weakness on one side of the body) to loss of consciousness. The three previous cases by Khosla et al., also carried out radiological examinations in the form of CT-SCAN and/or MRI to support the diagnosis of cerebral toxoplasmosis.[3,6,13,15]

PLHIV who were on WHO stage 3 or 4 and/or CD4 count <200 cells/μL (including pregnant women) should be recommended to receive cotrimoxazole prophylaxis. On April 8, 2021,

the patient's CD4 test results were 4 cells/μL and could be categorized into the severe immunosuppression group requiring immediate ARV therapy and cotrimoxazole. Adult patients with HIV infection who are about to start ARV therapy and have a CD4 count <200 cells/μL are recommended to be given cotrimoxazole treatment two weeks before starting ARV therapy. In this case, patients had been given cotrimoxazole 1x960 mg on April 6, 2021, before initiation of ARV 2 weeks later. This delay in administration time is useful for reducing or preventing overlapping side effects between cotrimoxazole and ARV therapy, considering that many ARV combinations have the same side effects as cotrimoxazole such as nausea, vomiting, hepatotoxicity, and others. In two out of three cases reported by Khosla et al., the patient was also given ARVs as HIV therapy.[3,4,13,15]

As previously mentioned, this patient meets the WHO-defined criteria for stage 4 HIV disease or advanced HIV disease (AHD). Patients with AHD are at a high risk of death, and their mortality rate increases as their CD4 cell count decreases, even after initiating ARV treatment. Typically, patients with AHD have a life expectancy of 2 years if they are on ARV. One of the most prevalent causes of severe illness and death is coinfection-related brain damage, as reported in this case. However, the prognosis for patients who are treated with ARV agents and achieve a CD4 count greater than 500 is similar to that of someone without HIV. On the other hand, those with untreated AIDS usually have a life expectancy of about 1 to 2 years after their first opportunistic infection. The goal of initiating ARV treatment for this patient is to improve the immunity system by increasing their CD4 count and may changing their status from AIDS to HIV.[3,15]

Treatment of toxoplasmosis includes management of induction therapy followed by maintenance therapy to prevent recurrence in patients with CD4 <200 cells/mm^3. Standard therapy is an induction pyrimethamine loading dose of 100 mg, followed by 50 mg maintenance therapy plus sulfadiazine 1000 mg every 6 hours or clindamycin 600 mg every 6 hours for 6 weeks. To reduce the toxicity of pyrimethamine to the bone marrow, a folic acid supplementation of 2 to 4 mg/day can be given.[5,12,16,17]

The monitoring of liver function, creatinine serum, and blood counts was carried out in this case, for the side effects of hepatotoxicity, nephrotoxicity, and anemia are the common association the pyrimethamine and ARV treatment.[3,12,17,18]

A phase II randomized trial involving 59 patients with cerebral toxoplasmosis concluded that after 6th weeks of treatment, respectively 70% versus 65% of patients in the pyrimethamine-sulfadiazine versus pyrimethamine-clindamycin group both showed a partial or complete clinical response. Pyrimethamine and Clindamycin therapy has been given to patients since the patient was diagnosed with toxoplasmosis cerebral on April 5, 2021. The patient received Clindamycin 4x600mg and pyrimethamine at a loading dose of 1x200mg, followed by a maintenance dose of 1x75 mg. The dose of pyrimethamine is appropriate since the patient's weight is <60 kg. Folic acid has been given to reduce the toxicity of pyrimethamine to the bone marrow with the dose obtained is 3x1 mg (3 mg per day).[5,17,19]

The mechanism of pyrimethamine itself is to work synergistically by inhibiting T gondii proliferation and survival through inhibition of the folate metabolic pathway. Pyrimethamine inhibits dihydrofolate reductase and dihydropteroate synthase and consequently blocks the synthesis of tetrahydrofolate which is required by the parasite for DNA synthesis. This becomes the reason why it is necessary to add folate supplementation in patients receiving pyrimethamine therapy.[17,18,20]

Patients still received pyrimethamine and clindamycin therapy until the patient was discharged from the hospital (May 3, 2021). The patient experienced an improvement in clinical condition with improved consciousness and hemoglobin level which was initially 8.3 g/dL to 10.4 g/dL just before leaving the hospital. Cerebral toxoplasmosis was associated as a factor causing other complications in the form of serum sodium depletion as experienced by the patient, although complaints of vomiting and diarrhea could also be the reason.[9,10,21]

Hyponatremia is reported frequently in HIV/AIDS patients. In Xinjiang, China, 40% of HIV/AIDS patients experience hyponatremia, especially in the presence of opportunistic infections in the CNS called toxoplasmosis. Toxoplasmosis can cause infections in the brain, leading to brain edema or affecting its blood flow. The edema in this case occurs in the right frontal-parietal-occipital region, putting pressure on the right lateral ventricle and the third ventricle area. This can interfere with the hypothalamus, a region located in the ventral brain above the pituitary gland and below the third ventricle, which plays a vital role in coordinating the

endocrine system. The hypothalamus produces hormones by receiving signals from various parts of the brain, including ADH, which regulates fluid balance by promoting water reabsorption in the kidneys. Unfortunately, the disruption of the hypothalamus can lead to an excessive release of ADH, known as SIADH, resulting in the significant reabsorption of water in the body. This can dilute the sodium electrolytes increasingly in the bloodstream, leading to recurrent low sodium concentrations or hyponatremia.[9,21]

In this case, the management of hyponatremia was using an infusion of 3% NaCl 500 mL/24 hours for two days. In the beginning, the patient's serum sodium value was 117 mmol/L, and the dose of 3% NaCl was 0.5–1.0 mL/kg/hour. For patients with a weight of 40 kg, the 3% NaCl needed in 24 hours was 480–960 mL, the dose given to the patient was appropriate. After being given 3% NaCl for two days, there was an increase in serum sodium to 135 mmol/L. Six days later, the results of the examination of sodium levels returned to show hyponatremia values (123 mg/L). If the administration of 3% NaCl is inadequate, it is necessary to add Vaptan therapy.[21,22]

Tolvaptan is a vasopressin-2 (V2) receptor antagonist and is licensed for use in euvolemic and hypervolaemic hyponatremia, not be used in hypovolaemic hyponatremia as it works by blocking the V2 receptors at the renal collecting duct, then causes a reduction of water reabsorption which can increase the clearance of free water without sodium loss. Tolvaptan demonstrated superiority to placebo in the treatment of Chinese SIADH patients with hyponatremia by elevating serum sodium concentration with an acceptable safety profile. The patient was given Tolvaptan for two days at a dose of 15 mg/day. The dose given to the patient was appropriate as much as 10–40 g/day. After the administration of tolvaptan, the patient's serum sodium value was 137 mmol/L. It is not recommended to increase serum sodium levels in hyponatremia patients too quickly or drastically (maximum of 10 mmol/L on day 1 and 8 mmol/L on the second day) due to osmotic demyelination of the CNS.[9,21,22]

Based on the clinical and supporting examinations of the patient discussed, the diagnosis of HIV stage 4 with cerebral toxoplasmosis coinfection and complications of SIADH is established. Stage 4 HIV cases require multiple steps of treatment. Firstly, Pyrimethamine-clindamycin is used to treat cerebral toxoplasmosis while monitoring consciousness. Secondly, Tenofovir, Lamivudine, and Evafirenz are used for antiretroviral therapy for HIV while monitoring CD4 counts and clinical condition. Additionally, NaCl 3% and tolvaptan are administered for hyponatremia correction while monitoring sodium levels. The treatment of these conditions requires the collective expertise and effort of multiple specialists to oversee drug therapy, ensure effective treatment outcomes, and promptly address any side effects.

References

1. UNAIDS. Global HIV Statistics. Fact Sheet 2021. 2021;(June):1–3.
2. Kemenkes RI. Profil Kesehatan Indonesia. Jakarta: Kementrian Kesehatan Republik Indonesia; 2021. Available from: http://www.kemkes/go.id.
3. WHO. Consolidated guidelines on HIV prevention, testing, treatment, service delivery and monitoring: recommendations for a public health approach. Geneva: World Health Organization; 2021. p. 594.
4. Kemenkes RI. Pedoman Nasional Pelayanan Kedokteran Tata Laksana HIV. Jakarta: Kementrian Kesehatan Republik Indonesia; 2019. Available from: https://kemkes.go.id/id/pnpk-2019---tata-laksana-hiv.
5. Vidal JE. HIV-Related cerebral toxoplasmosis revisited: current concepts and controversies of an old disease. J Int Assoc Provid AIDS Care. 2019;18:2325958219867315. doi:10.1177/2325958219867315
6. Luma HN, Tchaleu BCN, Mapoure YN, Temfack E, Doualla MS, Halle MP, et al. Toxoplasma encephalitis in HIV/AIDS patients admitted to the Douala general hospital between 2004 and 2009: A cross sectional study. BMC Res Notes. 2013;6(1):2–6.
7. Shu Z, Tian Z, Chen J, Ma J, Abudureyimu A, Qian Q. HIV/AIDS-Related hyponatremia: an old but still serious problem. Ren Fail. 2018;0(0):68–74. doi: 10.1080/0886022X.2017.1419975
8. Krajewska M, Kruczkowska A, Kusztal MA, Królicka AL. Hyponatremia in infectious diseases—a literature review. Int J Environ Res Public Health. 2020;17(15):1–16.
9. Xu L, Ye H, Huang F, Yang Z, Zhu B, Xu Y, et al. Moderate/Severe Hyponatremia Increases the Risk of Death among Hospitalized Chinese Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome Patients. PLoS One. 2014;9(10):e111077.

doi:10.1371/journal.pone.0111077
10. Musso CG, Belloso WH, Glassock RJ. Water, electrolytes, and acid-base alterations in human immunodeficiency virus infected patients. World J Nephrol 2016; 5(1):33–42
11. Madariaga H, Kumar A, Khanna A. A rare mechanism of hyponatremia in HIV disease. Am J Case Rep. 2015;16:707–10.
12. Klatt EC. Pathology of HIV/AIDS Version 28, 33rd ed. J Gen Virol. 2022;91(PT 1):1–545.
13. Khosla A, Singhal S, Jotwani P, Kleyman R. Cerebral toxoplasmosis as the initial presentation of HIV: A Case Series. Cureus. 2022;14(3):1–8.
14. Urano K, Ishibashi M, Matsumoto T, Ohishi K, Muraki Y, Iwamoto T, et al. Impact of physician-pharmacist collaborative protocol-based pharmacotherapy management for HIV outpatients: A retrospective cohort study. J Pharm Health Care Sci. 2020;6:9. doi:10.1186/s40780-020-00165-9.
15. Koshy AA, Harris TH, Lodoen MB. Cerebral toxoplasmosis. Toxoplasma Gondii. Weiss LM, Kim K, editors. Chapter 24 - Cerebral toxoplasmosis. 3rd ed. United States: Academic Press; 2020. p. 1043–73.
16. Dunay IR, Gajurel K, Dhakal R, Liesenfeld O, Montoya JG. Treatment of toxoplasmosis : historical perspective animal. Clin Microbiol Infect. 2018;31(4):1–33. doi:10.1128/CMR.00057-17
17. Ben-Harari RR, Goodwin E, Casoy J. Adverse event profile of pyrimethamine-based therapy in toxoplasmosis: a systematic review. Drugs R D. 2017;17(4):523–44. doi:10.1007/s40268-017-0206-8
18. Wondifraw Baynes H, Tegene B, Gebremichael M, Birhane G, Kedir W, Biadgo B. Assessment of the effect of antiretroviral therapy on renal and liver functions among HIV-infected patients: a retrospective study. HIV AIDS (Auckl). 2016;9:1–7. doi:10.2147/HIV.S120979
19. Konstantinovic N, Guegan H, Stäjner T, Belaz S, Robert-Gangneux F. Treatment of toxoplasmosis: Current options and future perspectives [published correction appears in Food Waterborne Parasitol. 2020 Dec 15;21:e00105]. Food Waterborne Parasitol. 2019;15:e00036. doi:10.1016/j.fawpar.2019.e00036
20. Elsheikha HM, Marra CM, Zhu XQ. Epidemiology, Pathophysiology, diagnosis, and management of cerebral toxoplasmosis. Clin Microbiol Rev. 2020;34(1):e00115–19. doi:10.1128/CMR.00115-19
21. H, Badireddy M. Hyponatremia. [Updated 2023 Jun 14]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK470386/.
22. Chen S, Zhao JJ, Tong NW, Guo XH, Qiu MC, Yang GY, et al. Randomized, double blinded, placebo-controlled trial to evaluate the efficacy and safety of tolvaptan in Chinese patients with hyponatremia caused by SIADH. J Clin Pharmacol. 2014;54(12):1362–7. doi:10.1002/jcph.342

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Unusual Lifespan of Edwards' Syndrome in COVID-19 Era

Filla Reviyani Suryaningrat, Devatri Hudayari, Natasha Amalda, Wan Rita Mardhiya, Fiva Aprilia Kadi, Aris Primadi, Tetty Yuniati

Neonatology Division, Department of Child Health, Faculty of Medicine Universitas Padjadjaran
Dr. Hasan Sadikin General Hospital Bandung, Indonesia

Abstract

Edwards' syndrome is caused by an extra chromosome on the 18th chromosome. Patients with this diagnosis have median survival of only 3–14.5 days, with the majority of infants with full trisomy 18 type die before or shortly after they are born. There is only supportive treatment available for Edwards' syndrome. During the COVID-19 pandemic, providing health services was challenging, leading to less than optimum treatments added by patients' reluctance to visit healthcare facilities due to the fear of COVID-19 transmission. This case study presented an infant with Edwards' syndrome with major malformation. The patient had several organ problems, was admitted to the NICU, and continued with home care for monitoring for 6 months. Patient then died at 9 months of age. Maintaining the best quality of life for this patient was very challenging for parents and healthcare providers. Since the management for this type of sydnome is more palliative, good education, information, and psychosocial supports for the parents are needed to prepare them with the worst conditions, which was especially more challenging during the COVID-19 pandemic.

Keywords: Covid 19, full trisomy, survival, trisomy 18

Introduction

Edwards' syndrome or trisomy 18 was discovered by John H. Edwards in 1960, also known as autosomal aneuploidy chromosomal disorder. It is caused by an extra chromosome on the 18th chromosome and is the second most common genetic disorder after trisomy 21.[1,2] Patients with Edwards' syndrome have some characteristics in physical appearance, which may be accompanied by congenital heart defects, and abnormalities in the brain, gastrointestinal, and kidney.[3,4] The incidence varies from 1/2500 to 1/7000 live births, more often in girls (about 80%) compared to boys.[1,2]

Children with trisomy 18 present with a range of congenital abnormalities, including mild to severe physical deformities, psychomotor impairment, cognitive deficits, and a high risk of intrauterine or postnatal death.[5] This current study reports a median survival of 3 to 14.5 days for infants with full trisomy 18, with the majority dying before or shortly after birth..[2,5]

Management is multidisciplinary based on the clinical manifestations, the decision of parents to care for the baby, ethical issues related to the high mortality rate, and the disabilities that arise if the baby survives.[2] The most common cause of death in these cases is heart problems and respiratory disorders.[2]

The COVID-19 pandemic situation has had an impact on restrictions in all routine health services, including the maternal and newborn health service sector. The impacts include a decrease in visits by pregnant women to healthcare facilities, issuance of recommendations for postponing pregnancy check-ups and classes for pregnant women, and limited health resources both in terms of personnel and facilities. Maternal and newborn health services are one of the services affected in terms of access and quality.[6]

In this case, we are about to discuss a baby with Edwards' syndrome who was born in the era of the COVID-19 pandemic, there are restrictions in various aspects of life including access to health services. The real impact of the COVID-19 pandemic, in this case, is that the health services provided to patients are not optimal. However,

Corresponding Author:
Fiva Aprilia Kadi
Neonatology Division, Department of Child Health, Faculty of Medicine Universitas Padjajaran/Dr. Hasan Sadikin General Hospital Bandung, Indonesia
Email: fiva.kadi@unpad.ac.id

this patient's life span can still last longer.

Case

A 33-day-old baby boy was referred to Dr. Hasan Sadikin General Hospital (RSHS) as a tertiary health care for West Java, Indonesia located in Bandung, with the diagnosis of pneumonia, ventricular septal defect, patent ductus arteriosus, heart failure, 36 weeks preterm infant, low birth weight, intrauterine growth restriction, cholestasis jaundice, bacterial conjunctivitis oculi sinister and microcephaly. The baby was given supplemental oxygen therapy at 0,5 liters per minute due to respiratory distress.

Table 1 Six Months Monitoring

Variable	Medical Problems
1st Month Chronological age: 4 months, 9 days Correction age: 3 months, 9 days	**Medical Problems,** The patient was unresponsive to calls, still using oxygen and fed by OGT sucking. Anthropometric status was underweight and short stature, developmental screening suspected intellectual disability. The patient had not been immunized. The parents were worried about the patient's growth and development and the COVID-19 pandemic. The parents were being educated about their concerns and how to stimulate development.
2nd Month Chronological age: 5 months, 9 days Corrected age: 4 months, 9 days	**Medical Problem,** Patient sometimes turned towards the sound. The patient began side-turning. Body weight has increased, the development began to progress. Oromotor training was done and OGT was replaced, advised to control polyclinics including ENT, eye, nutrition, neuropediatrics, social development and pediatrics, cardiology, endocrinology.
3rd Month Chronological age: 6 months, 9 days Corrected age: 5 months, 9 days	**Medical Problems,** The weight has increased; The patient received oromotor training and OGT nutritional fulfillment, OGT replacement, and monitoring oxygen therapy at home
4th month Chronological age: 7 months, 19 days Correction age: 6 months, 19 days	**Medical evaluation,** The patient responded to calls, sometimes smiled. The patient made a cooing sound. The patient paid attention to the sounding rattle. The patient has not received DPT, HiB, hepatitis B, and Polio immunization. The patient was no longer underweight but still short stature Patient received 135 ml/3-hour formula milk (723 kcal) Control to pediatrician Nutrition: The weight has increased, however, not on target, body length increase was not significant, and short stature was suspected to be related to the syndrome. The development began to progress, the patient began to respond to the sound
5th Month Chronological age: 8 months 9 days Correction age: 7 months 9 days	**Medical evaluation** Weight has increased however, not on target, body length increase was not significant **Plans** Formula milk plan was increased to 150 ml/3 hours (804 kcal) Control to polyclinics Immunization of DPT, HiB, hepatitis B, and Polio 2. Chest X-ray and laboratory examination were planned
6th Month Chronological age: 9 months Correction age 8 months	**Medical Problem,** The patient was still using oxygen at 1 LPM nasal cannula and getting nutrition through OGT. The patient was still a short stature **Medical evaluation,** The patient-controlled to a pediatrician and was advised to undergo physiotherapy and eye and ear examinations

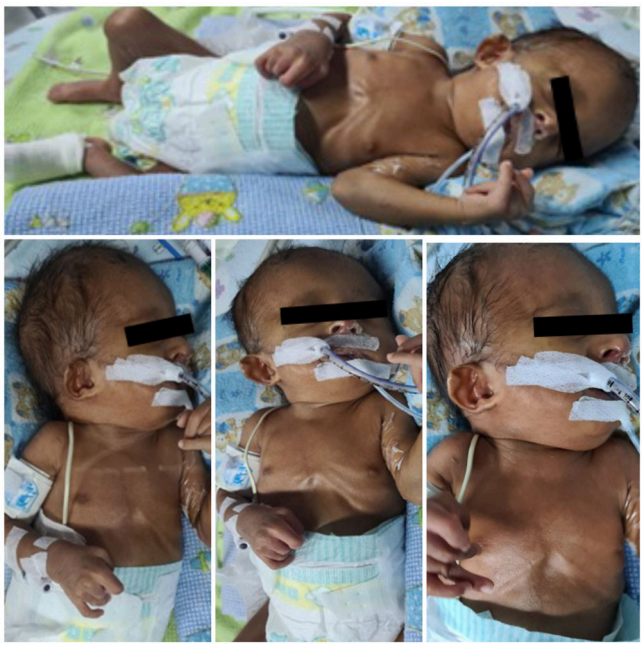

Figure 1 The Condition of the Baby at the Time of Being Treated at RSHS

Actual weight 2060 grams, length 45.5 cm with a head circumference of 32.5 cm. Physical examination showed facial dysmorphic micrognathia, strabismus, low-set ears, and hand clenched with abnormal fingers (Figure 1). On the fourth day of treatment, the patient experienced respiratory failure and was transferred to the Neonatal Intensive Care Unit (NICU).

The patient was born at a secondary healthcare hospital in Bandung, West Java, since the family resides in Bandung. The baby boy was born to a P4A0 mother by cesarean section with the indication of Intra Uterine Growth Restriction (IUGR) and high-risk mother (aged 42 years), birth weight of 2050 grams, birth length of 42 cm, head circumference 32 cm, APGAR score 1 minute and 5 minutes were 6/8 with no history of bluish at birth. The patient was referred to another secondary healthcare hospital in Bandung with more comprehensive tools for intensive care. On the 22nd day of treatment, the patient was referred to RSHS for further treatment.

Chest X-ray results showed right pneumonia

Figure 2 Patient at 8 Months of Age

with a differential diagnosis of right superior lobe atelectasis and cardiomegaly. Chest X-rays suggested bilateral pneumonia and cardiomegaly. The echocardiography showed a large perimembrane ventricular septal defect and a tiny patent ductus arteriosus. Ultrasound showed a right inguinal hernia and mild hepatomegaly. A head ultrasound examination revealed benign external hydrocephalus. An ultrasound examination of the testicles revealed a right inguinal hernia with a right hydrocele. Analyze of chromosomes that were 47XY + full trisomy 18.

The patient's respiratory function was supported with a mechanical ventilator. Multidisciplinary care involved several specialties, including Cardiology, Gastrohepatology, Respirology, Tropical Infectious Disease, Endocrinology, Neuropediatrics, Nutrition and Metabolic Disease, ENT, and Nutrition and Metabolic Disease.

The final diagnosis included Edwards syndrome, respiratory failure, pneumonia, suspected bronchopulmonary dysplasia, congenital CMV infection (manifesting as pneumonia, jaundice, microcephaly, failure to thrive, and intrauterine growth restriction), a large perimembranous ventricular septal defect (VSD), a tiny patent ductus arteriosus (PDA), mild heart failure, marasmus, short stature, and inguinal hernia.

After clinical improvement, the patient was discharged on the 43rd day of treatment using an orogastric tube (OGT) and oxygen 1 liter/ minute via nasal cannula.

The patient was visited by nurses for a home visit every 2 weeks, the monitoring results for 6 months are described in Table 1.

Monitoring for 6 months was carried out with home visits, parents did not bring their children for treatment control because they were worried about the increase in Covid-19 cases, therefore vision and hearing screening had not been carried out. During monitoring there was an increase in body weight, but still in short stature. The condition of the patient at 8 months of age is shown in Figure 2. At the age of 9 months, the patient was taken to the nearest hospital, and according to the doctor, the patient was death on arrival.

Discussion

The patient was the fourth child of four and was born by cesarean section. Since birth, the patient had shortness of breath and congenital heart disease. The patient was treated in NICU using a ventilator. Chromosomal analysis revealed Edwards' syndrome with full trisomy 18. The patient returned home with improvement, however, he was still on oxygen and OGT. Nurses made home visits to help care for patients every two weeks, the OGT was routinely changed. The family cared for and loved the patient with great affection.

Edwards' syndrome or trisomy 18 is caused by the presence of an extra chromosome on chromosome 18 and has various clinical manifestations with abnormalities on a scale of mild to severe.[5] Trisomy 18 has three types: full trisomy 18, partial trisomy 18, and mosaic trisomy 18, with 95% of all cases being full trisomy 18, where every cell in the body has an extra chromosome. Partial trisomy 18 and mosaic trisomy 18 are very rare.[7]

International guidelines for resuscitation and management of neonates with malformations recommend providing specialized palliative or supportive care and always emphasize that decisions must be made with the medical team and respect for parental autonomy.[8] The majority of infants with full trisomy 18 die before or shortly after they are born, some that survive will have severe cognitive impairment. Abnormalities include congenital heart defects, limb abnormalities, stunted fetal growth, and meningomyelocele.[9]

The clinical manifestations were facial dysmorphic micrognathia, low set ear, strabismus, hand clenched with abnormal fingers, ventricular septal defect, patent ductus arteriosus, right inguinal hernia, right hydrocele, and hydrocephalus. This patient had experienced severe clinical conditions at the beginning of life due to the abnormalities, nevertheless, after intensive treatment, clinical improvement was obtained, therefore, treatment could be continued at home. Parents were worried about home care but began to understand and accept the clinical condition after being explained slowly. They were willing to give the best to the patient; however, they were hampered by the pandemic.

A case report was obtained regarding 152 patients with Edward's syndrome. The male:female ratio was 1.3:1. More than half of the cases found that the parents' age was less than 30 years and were primigravida.[7] The most common disorders were cardiovascular, extremities, urinary tract, head, neck, GI tract, and genitalia. The most typical cardiovascular abnormalities

are VSD, atrial septal defect, and PDA. Common extremity abnormalities were abnormalities of the calcaneovalgus, hip abduction, and abnormalities of the fingers. The most common eye disorders were microphthalmos, epicanthal folds, and ocular hypertelorism. Regarding the ear, abnormalities were low set ear. Micrognathia and a short neck were also usual. In the GI tract, diaphragmatic hernia, umbilical hernia, and pyloric stenosis were often found. In the urinary tract often found hydronephrosis, hydroureter of the posterior urethral valve. In the testes, an undescended testis was often found.

Prenatal diagnosis using fetal DNA taken from maternal serum can identify abnormalities in the fetus at 10 weeks gestation. There is no actual data about the ratio of chosen termination compared to palliative care.[9] Congenital heart disease can be detected at the age of nine days, due to the poor prognosis, cardiology intervention was not performed.

Higher mortality rates were reported in the first week of life and the longest survival rate of most premature infants was 39 days. A case study mention that female carrier are more likely to survive longer, particularly with the mosaic type. Nonetheless, they had severe dependence and psychomotor retardation.[11] However, this study found an unusual pattern because the patient gender is male with full trisomy type and premature survived until 9 months.

The life expectancy of this case might increase due to the fast initial treatment since the baby was immediately given a mechanical ventilator when the respiratory failure happened. This is related to the three major causes of sudden death in Edwards syndrome: neurological instability, cardiac failure, and respiratory failure.[12]

The patient also receives a lot of attention in the tertiary healthcare hospital, where there is a Multidiscipline involved to treat the patient, such as Cardiology, Gastrohepatology, Respirology, Tropical Infectious Disease division, Endocrinology, Neuropediatric, Nutrition and Metabolic Disease, ENT, and Nutrition and Metabolic Disease division. This is similar to a case study mentioning that an 8-year-old girl with Edward syndrome and mosaic type has been treated by a multidisciplinary medical team due to musculoskeletal, joint, neurological, metabolic, and cardiovascular complications that have limited her quality of life.[13]

About 40-70% of Children with disabilities, including trisomy, have feeding problems. The factors include neurological status, anatomical problems, difficulty breathing, or motor disorders in the mouth. The interaction of one or more of these factors causes developmental delays or disorders that ultimately lead to feeding problems.[14] In this patient, feeding and breathing problems were also found. During home care, this patient used OGT to achieve nutritional needs and oxygen for his breathing problems.

The Denver II screening found delays in four aspects including gross motor, fine motor, language, and personal-social. The CAT CLAMS examination resulted in a suspected intellectual disability. This was suitable with the literature stating that developmental delays generally occur in patients with Edward's syndrome, both psychomotor disorders and intellectual disabilities. Most cases have expressive language disorder and gross motor disorder. Patients are unable to walk on their own, but a small proportion by using assistive devices. However, developmental delays are predictable, and developmental assessments are mandatory. Medical intervention and rehabilitation programs are recommended to reduce the progression of the delays that occur.[15]

The COVID-19 pandemic has caused a decline in the number of infants and toddlers that went to health facilities. Parents were worried that the patient would catch COVID-19. Furthermore, regarding the Large-Scale Social Restriction Policy (PSBB) to stay at home to prevent COVID-19, likewise limiting health service activities in health facilities.[16] Initially, the parents were concerned about managing the patient's condition in the hospital. After several counseling sessions, they agreed to continue with home care, including bi-weekly visits from nurses. A case study indicates that managing Edwards syndrome also requires counseling for parents regarding survival, frequent complications, and a risk-benefit assessment to be evaluated during the first year of life.[13]

The COVID-19 pandemic has also caused a decline in the number of infants and toddlers that immunized. Myths and misinformation about COVID-19 vaccine added to the doubts. Health workers were also adjusted for service hours or shifted to COVID-19 emergency health facilities and the lack of personal protective equipment (PPE). Vaccination officers were also concerned about the risk of transmitting COVID 19 that may occur during immunization services.[16] The decline in immunization visits was also found in the United States. The government-imposed social distancing and quarantine had an impact on immunization visits. The Michigan

Care Improvement Registry (MCIR) conducted a cohort study of changes in immunization coverage for children aged 1, 3, 5, 7, 16, 19, and 24 months during the pandemic and the result was a decrease in immunization coverage at every age, except the Hepatitis B vaccine.[17] In this case, the patient only received immunization once, this was also due to the worry to take the patient to healthcare facilities.

In this study, we found several limitations; firstly, our unique single case may not represent other cases, constraining our ability to extend these conclusions to a broader context. The lack of a control group in our study design significantly weakens the definitive cause-and-effect relationship. Lastly, considering the patient predominantly resided outside the hospital setting, numerous aspects of their condition remained beyond our observational scope. These limitations underscore the need for cautious interpretation of our results and suggest the value of further, more extensive studies in this area.

Edwards' syndrome, or trisomy 18, is characterized by numerous congenital abnormalities requiring palliative management. Essential aspects of care include immediate initial treatment, multidisciplinary involvement, ongoing evaluation of growth and development, and counseling with parents. These measures may improve life expectancy in individuals with Edwards syndrome. Additionally, social support and mental and psychological preparation for parents are crucial, particularly during the COVID-19 pandemic, to help them cope with challenging conditions.

References

1. Katasi C. Edwards' syndrome: A case study. Indian J Cont Nsg Edn. 2019;20:18–21.
2. Cereda A, Carey JC. The trisomy 18 syndrome. Orphanet J Rare Dis. 2012;7:81. doi:10.1186/1750-1172-7-81
3. Bruns D. Caring for an infant with trisomy 18: A case study and guidelines. Clinical Nursing Studies. 2013;2(1):30.
4. Imataka G, Suzumura H, Arisaka O. Clinical features and survival in individuals with trisomy 18: A retrospective one-center study of 44 patients who received intensive care treatments. Mol Med Rep. 2016;13(3):2457–66. doi:10.3892/mmr.2016.4806
5. Kaulfus ME, Gardiner H, Hashmi SS, Mendez-Figueroa H, Miller VJ, Stevens B, et al. Attitudes of clinicians toward cardiac surgery and trisomy 18. J Genet Couns. 2019;28(3):654–63. doi:10.1002/jgc4.1089
6. Kementerian Kesehatan RI Direktorat Jenderal Kesehatan Masyarakat Direktorat Kesehatan Keluarga. Pedoman pelayanan antenatal, persalinan, nifas dan bayi baru lahir di era adaptasi kebiasaan baru. Jakarta: Kementerian Kesehatan RI; 2020. p. 2–3.
7. Arakeri S, Fatima U, Ramkumar KR. A case report of edward syndrome and review of 152 similar cases published in various journals. Sch J App Med Sci. 2014;22(c):749–51. doi: 10.36347/sjams.2014.v02i02.057
8. Duque JAP, Ferreira CF, Zachia SA, Sanseverino MTV, Gus R, Magalhães JAA. The natural history of pregnancies with prenatal diagnosis of Trisomy 18 or Trisomy 13: Retrospective cases of a 23-year experience in a Brazilian public hospital. Genet Mol Biol. 2019;42(1 suppl 1):286–96. doi:10.1590/1678-4685-GMB-2018-0099
9. Pallotto I, Lantos JD. Treatment Decisions for Babies with Trisomy 13 and 18. HEC Forum. 2017;29(3):213–22.
10. López-Ríos V, Grajales-Marín E, Gómez-Zambrano V, Barrios-Arroyave FA. Prolonged survival in Edwards syndrome with congenital heart disease: a case report and literature review. Medwave. 2020;20(8):e8015.
11. Bruns D, Campbell E. Twenty-two survivors over the age of 1 year with full trisomy 18: presenting and current medical conditions. Am J Med Genet A. 2014;164a(3):610–9.
12. Balasundaram P, Avulakunta ID. Edwards Syndrome. [Updated 2023 Mar 20]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK570597/
13. López-Ríos V, Grajales-Marín E, Gómez-Zambrano V, Barrios-Arroyave FA. Prolonged survival in Edwards syndrome with congenital heart disease: a case report and literature review. Medwave. 2020;20(8):e8015. doi:10.5867/medwave.2020.08.8015
14. FBruns D, Springer S. Feeding changes in children with trisomy 18. Topics in Clinical Nutrition. 2013;28:324–34.
15. Begum R. A Case report on edward syndrome. Clinical Case Reports. 2019;9:1–2.
16. Felicia FV, Suarca IK. Pelayanan imunisasi dasar pada bayi di bawah usia 12 bulan dan faktor yang memengaruhi di RSUD Wangaya Kota Denpasar selama masa pandemi

COVID-19. Sari Pediatri. 2020;22(3):139–45.
17. Santoli J, Lindley M, DeSilva M, Kharbanda E, Daley M, Galloway L, et al. Effects of the COVID-19 Pandemic on Routine Pediatric Vaccine Ordering and Administration- United States, 2020. MMWR Morb and Mortal Wkly Rep. 2020;15;69(19):591–3. doi:10.15585/mmwr.mm6919e2