RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v56.3281
Majalah Kedokteran Bandung. 2024;56(1):1–7

Majalah Kedokteran Bandung (MKB)

Received: March 9, 2023
Accepted: August 8, 2023
Available online: March 31, 2024

# Correlation of Hepatocyte Growth Factor Levels with Myopia Degree and Axial Length in Outpatient Myopic Adolescents

**Fithria Aldy,[1] Gema Nazri Yanni,[2] Jelita Siregar[3]**
[1]Department of Opthalmology Universitas Sumatera Utara, Medan, Indonesia
[2]Department of Pediatric Universitas Sumatera Utara, Medan, Indonesia
[3]Department of Pathology Clinic Universitas Sumatera Utara, Medan, Indonesia

**Abstract**

Myopia is the most common ophthalmic condition worldwide, with an estimated 22.9% of the population or 1.406 billion people affected. Studies of candidate gene-associated refraction abnormalities have also identified several genes that cause vulnerability myopia, including the hepatocyte growth factor (HGF) and hepatocyte growth factor receptor (MET) genes. The purpose of the study was to analyze the correlation between hepatocyte growth factor (HGF) levels, degrees of myopia, and long axial in adolescents with myopia treated as outpatients in Universitas Sumatera Utara Hospital. This study applied the cross-sectional analytical approach. The inclusion criterion for the participants was patients diagnosed as suffering from myopia by the Ophthalmology Outpatient Clinic of the Universitas Sumatera Utara Hospital. Sampling was performed consecutively with a minimum sample size of 25 patients in control group and 25 patients in the case group. This study revealed that the mean axial length of the right eye in the case group was 23.82 mm, with a significant difference in the axial length between the case and control groups. The mean HGF level in the myopia group was 510.49 ng/mL, which was not significantly different from the mean HGF level in the control or emmetropic group. The HGF levels were highest in the group of subjects with moderate myopia, with an average of 551.87 ng/mL, and differences in HGF levels were identified in different degrees of myopia. Thus, no significant correlation is identified between the HGF levels, axial length, and myopia degree.

**Keywords:** Axial length, hepatocyte growth factor, myopia

## Introduction

Myopia is the most common ophthalmic disease in the world, with an estimated 22.9% of the population affected, or 1.406 billion people.[1,2] Prevalence and incidence of myopia throughout the world have increased dramatically in the past 50 years, especially in East Asia and Southeast Asia. In 2050, the amount will reach 4.8 billion.[3] The prevalence of myopia in Indonesia is estimated at 26.1%.[4] Report from Indonesia Research Health Basis (RISKESDAS) 2013 shows prevalence abnormality refraction that has been corrected in Indonesia by 4.6% and in North Sumatra by 4.0%.[5] Estimated interference vision in preschool children will increase by 26% by 2060, with error refraction that doesn't correct as many as 69% of cases.[6]

The underlying mechanism of myopia is a molecular change on the sclera caused by the remodeling process. During this process, depletion and changes in the architecture of the sclera cause enhancement and elongation axial. Remodeling sclera is generated from changing the composition and content of collagen. Hepatocyte growth factor (HGF) and its receptor (MET), which are highly expressed in the retina, epithelium pigment, and choroid, presumably play a key role in matrix metalloproteinase and blocker network track metalloproteinases and may also play a role in remodeling the sclera, lengthening the axial, and developing myopia. Studies on candidate gene association abnormality refraction have also identified several genes that cause vulnerability to myopia, including These include the hepatocyte growth factor receptor (MET) gene and the HGF gene.[7,8]

Identification of molecular and genetic variables in myopia might provide insight into the diagnostic, prediction, treatment, and

**Corresponding Author:**
Fithria Aldy,
Department of Opthalmology Universitas Sumatera Utara, Medan, Indonesia
Email: fithria.aldy@yahoo.com

prevention of myopia, especially in adolescents, in whom sight would be vital to ensuring a productive quality of life. A study on HGF might be a pioneering study that could describe the pathophysiology, treatment, or diagnosis of myopia.

## Methods

This study uses an analytic observational research method with a cross-sectional approach. The study was done in the Eye Polyclinic, Pediatric Ophtalmology Division, Universitas Sumatera Utara Hospital. This study lasted from March 2022 until June 2022, when the sample size was reached.Population case study This is because all patients in this study had a diagnosis of myopia, whereas the affordable population was patients with myopia Opthalmology Polyclinic of the Pediatric Opthalmology Division in Universitas Sumatera Utara Hospital.

The patient will undergo inspection refraction, examination biometry, and taking blood at Universitas Sumatera Utara Hospital. The sample size was determined by the correlation test method, i.e., all patients who visited the Ophtalmology Polyclinic at Universitas Sumatera Utara Hospital. A sample size formula for an analytical study is used to calculate the sample size. Through the formula, we observed that to gain statistical power, minimal samples of 25 patients per group were necessary. The sample size formula used for correlative analysis research will be used, and samples will be collected using the consecutive sampling method until all the samples have been fulfilled.

Criteria for inclusion in the study This is 12–25 years old, with myopia with >-1.00 spherical equivalent -6.00 D and ready to becomes sample research. Criteria for exclusion from the study are: systemic disease inflammation, moderate consumption of systemic drugs (e.g., steroids, anti-inflammatory nonsteroidals, vitamins), infection on the eye, history of trauma to the eye, and amblyopia.

Ethical clearance has been obtained from the USU Health Research Ethics Committee through letter number 321/KEPK/USU/2022.

## Results

This study was followed by as many as 52 subjects. Subjects were divided into two groups, namely the case group and the control group. The case group included 26 myopic patients who visited the Ophthalmology Polyclinic, Pediatric Opthalmology Division, and Universitas Sumatera Utara Hospital. And the control group consisted of 26 subjects with normal eyes at the Universitas Sumatera Utara Hospital. All subjects have met the inclusion criteria. The average HGF level in the myopia group was 510.49 ng/mL (SD =90.76 ng/mL), with the lowest level of 337.81 ng/mL and the highest level of 676.08 ng/mL,

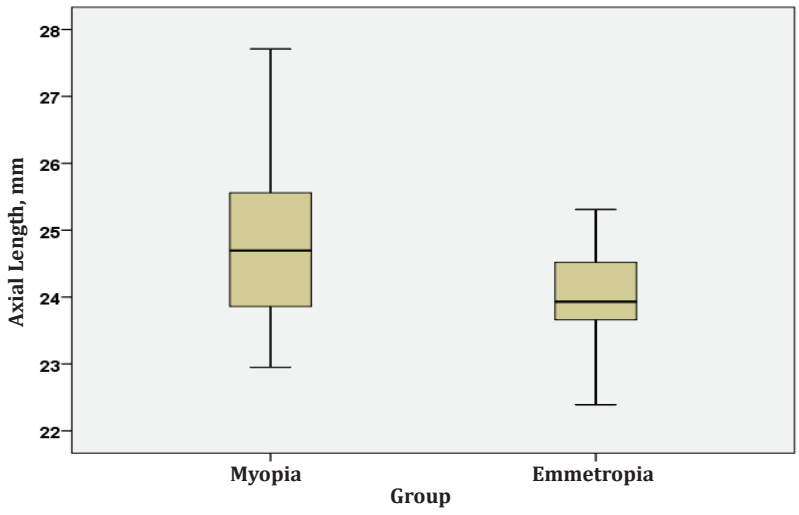


**Figure 1 Axial Length Boxplot Graph Myopia Group and Emmetropia Group**

**Table 1 Characteristics Demographics Subject with myopia and Emmetropia**

| Characteristics Demographics | Myopia (n=26) | Emmetropia (n=26) | p |
|---|---|---|---|
| Type Gender, n (%) | | | |
| Male | 10 (38.5) | 11 (42,3) | 0.777 [a] |
| Female | 16 (61.5) | 15 (57.7) | |
| Age, years | | | |
| Average (SD) | 21 (2.90) | 22.42 (0.5) | 0.115 [b] |
| Median (Min–Max) | 22 (12 – 23) | 22 (22 – 23) | |
| Axial Length, mm | | | |
| Average (SD) | 24.74 (1.18) | 23.98 (0.65) | <0.001 [b] |
| Median (Min–Max) | 24.70 (22.95–27.71) | 23.93 (22.39–25.31) | |
| HGF, ng/mL | | | |
| Average | 510.49 | 466.53 | 0.077* |
| SD | 90.76 | 102.05 | |
| Median (Min–Max) | 501.42 (337.81–676.08) | 492.94 (208–620.18) | |
| Degrees of Myopia | | | |
| Emmetropia | | 52 (50) | |
| Mild Myopia | | 30 (28.8) | |
| Moderate Myopia | | 14 (13.5) | |
| Severe Myopia; | | 8 (7.7) | |

[a] *Chi Square;* [b] *T Independent;* * Mann Whitney U Test

while the control group showed a lower value. with a mean of 466.53 ng/mL (SD=102.05 ng/mL), with the lowest level of 208 ng/mL and the highest level of 620.18 ng/mL. Using the Mann-Whitney test, the mean HGF levels in the myopia and emmetropia groups did not significantly vary from one another (p=0.077).

The demographic characteristics of the subjects are shown in Table 1.

The average HGF level in male subjects in the myopia group was 525.13 ng/mL, much higher than the HGF level in the emmetropic group, with an average of 432.28 ng/mL. Male subjects with myopia and emmetropia had significantly different amounts of HGF, according to the analysis's findings using the independent T test (p=0.006). However, HGF levels in the two study groups of female participants did not significantly differ from one another (p=0.955). All of these results can be observed in Table 2.

**Table 2 Differences in HGF Levels in Group Case and Control based on Type Sex**

| Type Sex | n | HGF, ng/mL Myopia | n | HGF, ng/mL Emmetropia | p* |
|---|---|---|---|---|---|
| Male | 20 | | 22 | | |
| Average (SD) | | 525.13 (86.21) | | 432.28 (115.28) | 0.006 [a] |
| Median (Min–Max) | | 509.6 (350.82–652.21) | | 471.83 (208–620) | 0.363 [b] |
| Female | 32 | | 30 | | |
| Average (SD) | | 501.35 (93.66) | | 491.65 (84.55) | 0.955 [c] |
| Median (Min–Max) | | 492.72 (337.81–676.08) | | 506.36 (337.41–614.88) | 0.049 [d] |

[a] T Independent (Myopia vs Emmetropia Male), [b] T Independent ([Male Myopia vs Girls Myopia), [c] Mann Whitney (Myopia vs Emmetropia Female), [d] Mann Whitney (Male Emmetropia vs Girls emmetropia)

**Table 3 HGF Levels Based on Degrees of Myopia**

| Degrees myopia | n | HGF level, ng/L | | p * | Posthoc [a] | | |
|---|---|---|---|---|---|---|---|
| | | Average (SD) | Median (Min- Max) | | Mild | Moderate | Severe |
| Emmetropia | 52 | 466.53 (102.05) | 492.94 (208-620.18) | 0.026 | 0.190 | 0.008 | 0.486 |
| Mild | 30 | 508.21 (96.76) | 493.83 (337.81-652.21) | | | 0.207 | 0.115 |
| Moderate | 14 | 551.87 (66.97) | 510.2 (492.02-676.08) | | | | 0.006 |
| Severe | 8 | 446.68 (69.24) | 428.96 (384.71-544.06) | | | | |

* Kruskal Wallis, [a] Mann Whitney

**Table 4 Correlation of HGF Levels with Axial Length and Degrees of Myopia**

| | Axial Length | Degrees of Myopia |
|---|---|---|
| **HGF levels** | p=0.127 | p=0.720 |
| | r=0.151 | r=0.036 |
| | n=104 | n=104 |

The average HGF level in male subjects in the myopia group was 525.13 ng/mL, much higher than the HGF level in the emmetropic group, with an average of 432.28 ng/mL. The results of the analysis using the independent T test showed that the myopia and emmetropia groups in male subjects had significantly different levels of HGF (p=0.006). The levels of HGF in the two study groups of female participants, however, did not differ significantly from one another (p=0.955). In the myopia group, there was also no difference in HGF levels between male and female subjects (p=0.363). In the emmetropic group, differences in HGF levels were found between male and female subjects (p=0.049).

Table 3 shows the distribution of differences in HGF levels based on the degree of myopia, which is shown in the table below.

HGF levels appear to be the highest on group moderate myopia subjects with a mean of 551.87 ng/mL (SD=66.97), followed by group myopia light with a mean of 508.21 ng/mL (SD =96.76 ng/mL), and the lowest on the group with severe myopia with a mean of 446.68 ng/mL (SD=69.24 ng/mL). Using the Kruskal Wallis test, it was discovered that there was a difference in HGF levels based on degrees of myopia (p=0.026). After a post hoc analysis, different HGF levels were significant among subjects with

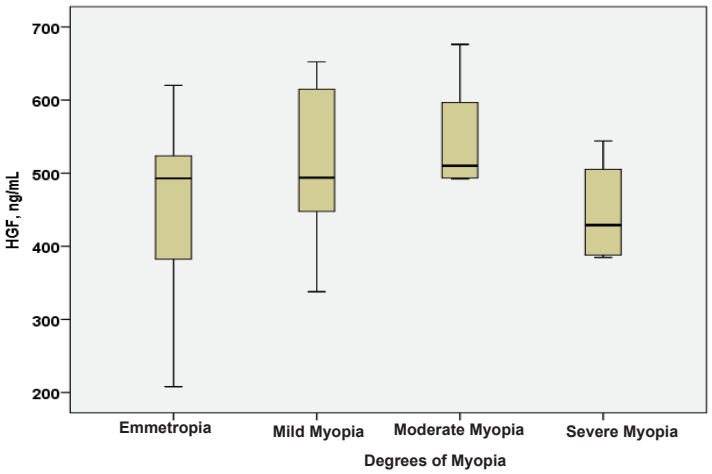


**Figure 2 Boxplot Graph of HGF Levels Based on Degrees of Myopia**

emmetropia with myopia medium (p=0.008) and among those with myopia currently and myopia weight (p=0.006). By using the Spearman correlation test, it was found that there was no significant correlation between HGF levels with axial length (p=0.127) and the degree of myopia (p=0.720). The result is shown in Table 4.

## Discussion

The study had performed to examine HGF and MET levels in myopic adolescents. Subjects in the case and control groups were mostly female, amounting to 16 people (61.5%) in the case group and 15 people (57.7%) in the control group. In the case group, the mean age was 21 years, with the youngest being 12 years old and the oldest being 23 years old. Meanwhile, in the control group with a mean age of 22.42 years, the youngest was 22 years old and the oldest was 23 years old. However, it must be underlined that there are more women than men, and there are studies that have found a sex relationship, especially among women, to the incidence of myopia. There could be two possible gender differences. The first is that biological factors determine differences. The second possibility is that they are influenced by social and behavioral factors.

One study determined that Orthodox Jewish boys who received an intensive religious education were more likely to be shortsighted than their sisters and other age groups who received a more secular education. Perhaps the high incidence and rate of myopia seen in the group of Orthodox men is a result of their distinct study habits, which cause them to utilize their accommodating eyes a lot more than other people do. Recent, extensive research on the incidence of myopia has found that girls are more likely to have the condition. The causes of sex differences are related to puberty and are influenced by diet, genetics, and the amount of close work. Girls start puberty earlier than boys do, which causes them to grow to their final height one or two years earlier. As a result, myopia becomes more prevalent. 10 The result is that there is no relationship between gender and the incidence of myopia, with the ratio seen being 54% (13756 people) in women and 46% (11707 people) in men, where the significance is 0.718, proving no significant relationship.[10]

Eyes with mild myopia were 30 (28.8%). There were 14 eyes with moderate myopia (13.5%) and 8 people (7.7%) with severe myopia. These results are consistent with research conducted in Indonesia, namely Avliwani and Aryani's[10] research that in fact mild myopia remains the majority of myopia cases followed by moderate and severe cases. This is further strengthened by Avliwani and Aryani's [10] research design which compares urban and rural populations where the results are the same that mild degrees remain the highest. This research can be compared with research conducted by researchers because the research sample came from the Polyclinic of Universitas Sumatera Utara Hospital while Avliwani and Aryani's research took samples from SMA Negeri 2 Medan and SMA Istiqlal Private in Deli Serdang where this shows both that in community and home settings pain degree of myopia does not change the proportion.[11]

Myopia sufferers are more commonly found in high school students in urban areas compared to high school students in rural areas. Genetics, prolonged reading, watching TV and using computers as risk factors for myopia degree. Therefore, refractive examination should be performed on children from primary school to protect myopia.

The mean axial length of the right eye in the case group was 23.82 mm (SD=1.69 mm) with the shortest axial length 21.44 mm and the longest 27.17 mm. The mean axial length OD in the control group was 22.28 mm (SD=0.72 mm) with the shortest axial length 20.89 mm and the longest 23.82 mm. Using the Mann Whitney test, a significant difference in axial length was found between the case and control groups for the right eye. These results are consistent with a study conducted by Damara and Ismail [11] where when tested the significance of the axial length and myopia degree using the Kruskal Wallis test, it was found that there was a significant relationship with mild myopia having an average axial length of 19.75 followed by moderate myopia 23.02 and severe myopia. 25,72. The results of the Kruskal Wallis test are p=<0.001.[12]

Basically, myopia occurs due to an increase in the axial length of the eyeball without being followed by changes in other refractive components. Likewise, changes in the refractive power of the cornea, lens and aqueous humor will cause myopia if it is not compensated for by changes in the axial length of the eyeball. Because it is contrasted with these pathogenesis explanations, this study reinforces that axial length will be more influential in the pathogenesis of myopia. The cause of myopia is a mismatch between optical power and the

axial dimensions of the eye. An imagine is distorted as a result of the light rays intersecting in front of the photoreceptor retinal layer. The primary determinants of eye axial length growth include genetic, environmental, and aging factors. According to recent studies, myopia is characterized by a longer axial length, a flatter corneal curvature, and a thinner lens.[13]

Myopia is a refractive error of the eye characterized by blurring of distant vision due to parallel rays of light entering the eye and hitting a focal point in front of the retina. Refractive errors such as myopia are actually caused by disturbances in the development of vision, such as alterations to the scleral component that make the axial length of the myopic eye significantly longer than that of the emmetropic eye. Changes in scleral composition are related to hepatocyte growth factor (HGF) and its cMET receptor, which regulate the matrix metalloproteinase (MMP) pathway, and tissue inhibitor MMP (TIMP), which plays an active role mediated by interactions. regulates scleral remodeling, which can lead to myopia. Mutations and polymorphisms in genes play an important role in altering the major structural components of the scleral extracellular matrix. The SNPs rs 3735520 and rs 12536657 are part of a polymorphism in the HGF gene that predisposes to myopia. In addition, the cMET gene polymorphism (MET 110703) is also involved in the pathogenesis of myopia. Polymorphisms in the HGF gene and its receptor, cMET, lead to an increase in MMP enzyme levels and a decrease in TIMP enzyme levels. This situation causes remodeling of the extracellular matrix that forms the sclera, resulting in myopia. This is because the anteroposterior diameter of the eyeball is elongated so that the incident light hits the preretinal focal point.[14]

HGF can be a promising finding because of the possibility of this gene playing a role in the scleral remodeling process in myopic conditions. There were significant correlations between HGF levels and various myopia severity levels in this study. HGF has been investigated by various studies to be able to increase the expression of MMP-2, which can cause degradation of various cells and tissues. In myopia, the specific mechanism of action of MMP-2 is not yet known, but it has been shown that MMP-2 in the sclera can cause extracellular matrix degradation and axial elongation which eventually develops into myopia. Therefore, HGF indirectly plays a role in the development of myopia. In vitro studies on animal samples have proven this.[15]

The study limitation was the study's single-center design, a multi-center study could better be able to capture a phenomenon. A larger sample size might also be more representative to capture HGF effect toward myopia pathophysiology however it must be noted that our sample size was calculated according to statistical rule and should have scientific power to some degree.

Myopia is a refractive eye disorder that is still common throughout the world. The mechanism underlying myopia is a molecular change in the sclera through the scleral remodeling process. Axial elongation rises as a result of sclera architecture alterations and sclera thinning throughout this process. Changes in the collagen content's composition lead to scleral remodeling. Hepatocyte growth factor (HGF) is thought to play a role in scleral remodeling, axial elongation, and myopia development. The results of this study were based on the degree of myopia, eyes with mild myopia were 28.8%, moderate myopia was 13.5%, and severe was 7.7%. The mean axial length of the right eye in the case group was 23.82 mm and there was a significant difference in axial length between the case and control groups. The mean HGF level in the myopia group was 510.49 ng/mL and did not significantly differ between the myopia and emmetropia groups. HGF levels appeared to be the highest in the group of subjects with moderate myopia with an average of 551.87 ng/mL and differences in HGF levels were found based on the degree of myopia. This study concluded that there was no significant correlation between HGF levels and axial length and myopia degree.

Further study should be done with multi-center and larger sample size to further recognize the role of HGF in myopic patients. In vitro or in vivo study might also describe the phenomenon in a more coherent manner.

## References

1. Chua J, Wong TY. Myopia-the silent epidemic that should not be ignored. JAMA Ophthalmol. 2016;134(12):1363–4. doi:10.1001/jamaophthalmol.2016.4008
2. Holden BA, Fricke TR, Wilson DA, Jong M, Naidoo KS, Sankaridurg P, et al. Global prevalence of myopia and high myopia and temporal trends from 2000 through 2050. Ophthalmology. 2016;123(5):1036–42. doi:10.1016/j.ophtha.2016.01.006
3. Chen CA, Lin PY, Wu PC. Treatment effect of posterior scleral reinforcement on controlling myopia progression: A

systematic review and meta-analysis. PLoS One. 2020;15(5):e0233564. doi:10.1371/journal.pone.0233564
4. Kalangi W, Rares R, Sumual V. Abnormalities refraction at the Eye Polyclinic, RSUP DR. RD Kandou Manado Period July 2014-July 2016', Journal Medical Clinic (JKK). 2020;1(1):83–91.
5. Hayatillah A. Prevalensi Miopia Dan Faktor–Faktor Yang Mempengaruhinya Pada Mahasiswa Program Studi Pendidikan Dokter UIN Syarif Hidayatullah Jakarta Tahun 2011 [Dissertation]. Jakarta: UINJKT; 2011. Available from: https://repository.uinjkt.ac.id/dspace/handle/123456789/25481.
6. Varma R, Tarczy-Hornoch K, Jiang X. Visual impairment in Preschool Children in the United States: demographic and geographic variations from 2015 to 2060. JAMA Ophthalmol. 2017;135(6):610–6. doi:10.1001/jamaophthalmol.2017.1021
7. Yang X, Liu X, Peng J, Zheng H, Lu F, Gong B, et al. Evaluation of MYOC, ACAN, HGF, and MET as candidate genes for high myopia in a Han Chinese population. Genet Test Mol Biomarkers. 2014;18(6):446–52. doi:10.1089/gtmb.2013.0479
8. Ding X, Zhang R, Zhang S, Zhuang H, Xu G. Differential expression of connective tissue growth factor and hepatocyte growth factor in the vitreous of patients with high myopia versus vitreomacular interface disease. BMC Ophthalmol. 2019;19(1):25. doi:10.1186/s12886-019-1041-1
9. Grzybowski A, Kanclerz P, Tsubota K, Lanca C, Saw SM. A review on the epidemiology of myopia in school children worldwide. BMC Ophthalmol. 2020;20(1):27. doi:10.1186/s12886-019-1220-0
10. Avliwani V, Amra AA. Comparison degree of myopia in Senior High School Students in Urban and Rural Area. Int J Sci Res Publ. 2018;8(5):387–91.
11. Damara S, Ismail A. Association of axial length and myopia degree: a retrospective study. Sriwijaya J Ophthalmology. 2022;5(2):141–4.
12. Burfield HJ, Carkeet A, Ostrin LA. Ocular and systemic diurnal rhythms in emmetropic and myopic adults. Invest Ophthalmol Vis Sci. 2019;60(6):2237–47. doi:10.1167/iovs.19-26711.
13. Fan Q, Wang H, Jiang Z. Axial length and its relationship to refractive error in Chinese university students. Cont Lens Anterior Eye. 2022;45(2):101470. doi:10.1016/j.clae.2021.101470
14. Arianto S. The role of SNP rs3735520 and rs 12536657 hepatocyte growth factor gene and MET + 110703 receptor gene cMET as factor predisposition myopia . Hermina Heal Sci J. 2021;1(1):1–6.
15. Li XJ, Yang XP, Wan GM, Wang YY, Zhang JS. Effects of hepatocyte growth factor on MMP-2 expression in scleral fibroblasts from a guinea pig myopia model. Int J Ophthalmol. 2014;7(2):239–44. doi:10.3980/j.issn.2222-3959.2014.02.09
16. Li XJ, Yang XP, Wan GM, Wang YY, Zhang JS. Expression of hepatocyte growth factor and its receptor c-Met in lens-induced myopia in guinea pigs. Chin Med J (Engl). 2013;126(23):4524–7.

RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v56.3307
Majalah Kedokteran Bandung. 2024;56(1):8–14

Majalah Kedokteran Bandung (MKB)

Received: March 30, 2023
Accepted: August 6, 2023
Available online: March 31, 2024

# Shallot (*Allium cepa L.*) Peel Infusion Ameliorates Kidney Histopathological Damages in Diazinon-Induced Wistar Rats

**Avie Baldana Bi'izzyk,[1] Dina Helianti,[2] Septa Surya Wahyudi,[3] Rosita Dewi[2]**
[1]Faculty of Medicine, University of Jember, Jember, Indonesia
[2]Histology Department, Faculty of Medicine, University of Jember, Jember, Indonesia
[3]Anatomic Department, Faculty of Medicine, University of Jember, Jember, Indonesia

**Abstract**

Diazinon, an organophosphate pesticide, is used extensively in agricultural sector. Consumption of agricultural products containing diazinon residue may lead to harmful health consequences. Among these is nephrotoxicity, which includes lipid peroxidation, that can damage the kidney. Flavonoids in shallot peel can scavenge free radicals, inhibit necrosis, and activate bone marrow-derived cells for cell regeneration. This study aimed to determine the correlation between shallot peel infusion (SPI) dose and kidney damage amelioration to establish the maximum effective dose of SPI to ameliorate kidney histopathological damage in diazinon-induced rats. This study was performed at the Pharmacology Laboratory, Faculty of Medicine, University of Jember, Indonesia, in April 2021, on 35 rats that were divided into 7 groups—normal, diazinon, and five treatment groups. Diazinon 40 mg/kgBW was administered on day 1-7, while SPI 125, 250, 500, 1,000, 2,000 mg/kgBW were administered on day 8–14 according to the treatment group. Kidney histopathological slides with hematoxylin-eosin (H.E.) staining were assessed using Kocoglu scoring and Kidney damage scores of the treatment groups were analyzed using Pearson test. The maximum effective dose was determined using regression test. The damages found in diazinon-induced rats were tubular degeneration, necrosis, and inflammation with a higher damage score than normal rats ($p<0.05$). Pearson test showed moderate correlation (coefficient -0.594). Higher SPI doses presented lower kidney damage scores, with 1,359 mg/kgBW being the maximum effective dose. SPI dose and the kidney damage amelioration are moderately correlated with a SPI maximum effective dose to ameliorate kidney damage in diazinon-induced rats of 1,359 mg/kgBW.

**Keywords:** Flavonoid, kidney, oxidative stress, pesticide, red onion

## Introduction

In agriculture sector, optimizing the usage of pesticide is an imperative aspect to do to increase the quality and quantity of agricultural products. Diazinon, an organophosphate pesticide, is extensively used by farmers.[1] WHO estimates that 1–5 million cases of organophosphate poisoning occur each year, 80% coming from developing countries. The consumption of agricultural products containing diazinon residues due to its excessive use causes hazardous effects on humans.[2] Diazinon has nephrotoxic effect related to renal function as a vital organ involved in the excretion of chemical substances.[3,4] The previous study revealed that diazinon at dose of 40 mg/kgBW caused the increase of blood urea nitrogen (BUN) serum level and kidney histopathological damage in Wistar rats.[5]Diazoxon, the active metabolite of diazinon, increases the level of free radicals which will bind to polyunsaturated fatty acids (PUFA) in cell membranes, including kidney nephrons, resulting lipid peroxidation.[6]

Natural ingredient is needed as an agent to repair tissue damage and prevent further organ damage. Shallot (Allium cepa L.), a horticultural commodity with almost evenly spread throughout Indonesia, contains antioxidant compounds i.e. flavonoids.[7–9] Shallot peel generally considered as waste contains 3–5 times higher flavonoid compounds compared to the tuber which is commonly consumed.[10] A phytochemical screening of shallot skin ethanol extract (SSEE) *using aluminium chloride colorimetric method* revealed that 1 g SSEE contained 228.1 mg QE total flavonoids.[11] Flavonoids neutralize free radicals, increase prostaglandin production,

**Corresponding Author:**
Dina Helianti,
Histology Department, Faculty of Medicine, University of Jember, Jember, 68121, East Java, Indonesia
Email: dinahelianti.fk@unej.ac.id

inhibit renal cell necrosis, and stimulate renal cell proliferation through activation of bone marrow-derived cells (BMDCs) leading to cell regeneration. It was discovered that engrafted BMDCs into the damaged cells plays an essential role in normal cell turnover to improve membrane integrity loss.[12]

Flavonoids are classified into the polar compounds dissolved in polar solvents such as water associated with their hydroxyl groups content.[13] Shallot peel can be formulated using water as a solvent called infusion which can be provided using common tools and materials as well as simple procedure for people to apply. The antioxidant effect of shallot peel infusion (SPI) has not been widely studied. This study needs to be carried out to determine correlation between the SPI dose and the amelioration of kidney histopathological damage and establish the maximum effective dose of SPI to ameliorate kidney histopathological damage in diazinon-induced Wistar rats.

## Methods

This study received ethical approval from The Ethical Committee for Research, Faculty of Medicine, University of Jember with reference number 1470/H25.1.11/KE/2021. This study was carried out at Pharmacology Laboratory Faculty of Medicine University of Jember, April 2021. The main material used was shallot peel Blue Lancor variety from Probolinggo, Allium cepa species L. var. ascalonicum Back. The other materials were rat feed pellets, liquid diazinon 600 g/L from farm shop Pasar Tanjung Jember, corn oil, aquadest, 10% neutral formalin buffer, xylol, paraffin, and hematoxylin-eosin (H.E.) dye. The shallot peels were cleaned and dried under the sunlight, subsequently crushed using a blender to produce a simplicial form. Simplicia were mixed with aquadest and heated in an infusion pan. The first pan was filled with water and heated and the second pan was placed on top of the first pan. To provide 20% SPI, as much as 10 g of shallot peel simplicia and 50 mL of distilled water were stirred and heated at 90°C for 15 minutes. The infusion is filtered using flannel and hot water is added to reach a volume of 50 mL.[14] The dilluted infusions were prepared using the serial dilution method to obtain 10% (5 g/50 mL), 5% (2.5 g/50 mL), 2.5% (1.25 g/50 mL), and 1.25% (0.625 g/50 mL) SPI concentrations.

The experimental animals used in this study were male white rats (Rattus norvegicus) Wistar strain, aged 2–3 months, and body weight of 150-250 g. As many as 35 male Wistar rats were randomly divided into normal group, diazinon group, and five treatment groups. On day 1-7, the normal group received 5 mL/kgBW corn oil, while the other groups received 40 mg/kgBW diazinon. On day 8-14, the normal and diazinon group received 10 mL/kgBW aquadest and the other group received SPI at doses of 125 mg/kgBW, 250 mg/kgBW, 500 mg/kgBW, 1,000 mg/kgBW, and 2,000 mg/kgBW. All treatments were administered orally.

Kidney histopathologic slides were made using paraffin method with H.E. staining and subsequently observed by anatomical pathologist using binocular microscope (Leica DM500) in five fields with 400X magnification. Kidney histopathological damage score of each rat was determined based on Kocoglu scoring (0: no kidney damage such as tubular degeneration, tubular cell necrosis, accumulation of cell debris in the lumen, tubular cast formation, tubular dilatation, and neutrophil infiltration), 1: ≤10% kidney damage, 2: 11-25% kidney damage, 3: 26–45% kidney damage, 4: 46–75% kidney damage; 5: 76–100% kidney damage).[15] Data were analyzed statistically using IBM SPSS Statistics 26. The average of kidney histopathological damage score of the normal and diazinon group were analyzed using independent T-test, while the treatment groups were analyzed using Pearson correlation test. The maximum effective dose was determined using regression test with quadratic curve.

## Results

Based on the result, the highest kidney histopathological damage score was found in diazinon group (Table 1). Independent T-Test

**Table 1 The Average of Kidney Histopathological Damage Score**

| Group | The Average of Kidney Histopathological Damage Score |
|---|---|
| Normal | 2.594 ± 0.556 |
| Diazinon | 3.413 ± 0.169 |
| SPI 125 mg/kgBW | 2.776 ± 0.233 |
| SPI 250 mg/kgBW | 2.658 ± 0.965 |
| SPI 500 mg/kgBW | 2.876 ± 0.309 |
| SPI 1,000 mg/kgBW | 2.079 ± 0.333 |
| SPI 2,000 mg/kgBW | 2.359 ± 0.373 |

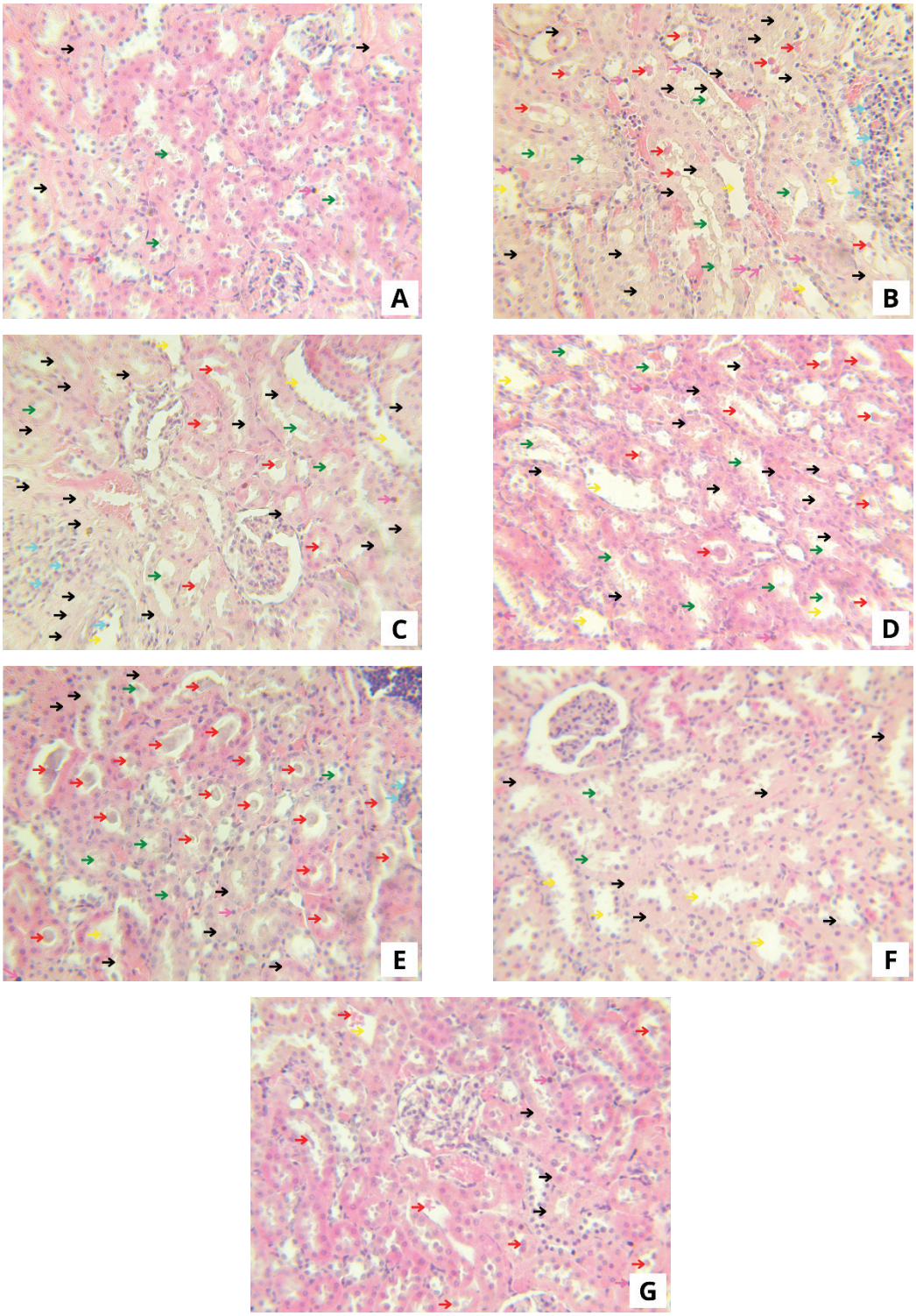


**Figure 1 Kidney Histopathological Damage with H.E. Staining (400X)**

A: normal group, B: diazinon group, C: SPI 125 mg/kgBW, D: SPI 250 mg/ kgBW, E: SPI 500 mg/ kgBW, F: SPI 1,000 mg/ kgBW, G: SPI 2,000 mg/ kgBW). Black arrow: tubular degeneration, pink arrow: tubular cell necrosis, green arrow: accumulation of cell debris in the lumen, red arrow: tubular cast, yellow arrow: tubular dilatation, and blue arrow: neutrophil infiltration

**Table 2 Pearson Correlation Analysis**

| Correlations | | Kidney damage score | Group |
|---|---|---|---|
| Kidney damage score | Pearson Correlation | 1 | -.594 |
| | Sig. (2-tailed) | | .002 |
| | N | 24 | 24 |
| Group | Pearson Correlation | -.594** | 1 |
| | Sig. (2-tailed) | .002 | |
| | N | 24 | 24 |

result between the normal and diazinon group showed a difference with significance value of 0.030 ($p<0.05$) indicating that diazinon is nephrotoxic.

The kidney histopathological damages found in microscopic observation were tubular degeneration, tubular cell necrosis, accumulation of cell debris in the lumen, tubular cast formation, tubular dilatation, and neutrophil infiltration (Figure 1). The result of Pearson correlation test showed moderate correlation with coeficient -0.594 (Table 2); the higher the SPI dose, the lower kidney histopathological damage score.

Based on the highest $R^2$ in regression test result, we used a quadratic curve with equation $y = 3{,}21 - 1.62\ .10^{-3}x + 5.96\ .10^{-7}x^2$ (Figure 2). The maximum effective dose of SPI is 1,359 mg/kgBW determined by calculating the x value from the derivative equation with y'=0.

## Discussion

Diazinon causes damage to the histological structure of kidney through various pathophysiological pathways. Diazoxon, the active metabolite of diazinon, inhibits acetylcholine esterase (AchE) enzyme activity, thereby increasing acetylcholine (Ach) levels in the body. ACh accumulation will increase $Ca^{2+}$ influx and nitric oxide (NO) production in endothelial cells.[6,16,17] These free radicals will bind to PUFA which are abundantly found in cell membranes, including kidney nephrons, resulting in lipid peroxidation.[6] The increase of $Ca^{2+}$ influx and NO production disrupts mitochondrial respiration and cause adenosine triphosphate (ATP) depletion. It will interfere ATP dependent ion transport which subsequently increase $Ca^{2+}$ influx and NO production. The excess of $Ca^{2+}$ influx will also increase the levels of calcium-dependent enzymes such as calpain, endonuclease, and ATP-ase which will trigger cell damage through necrosis.[16,17]

Tubular degeneration occurs due to the failure of the ATP-dependent ion pump on the cell membrane leading to disruption of fluid and ion

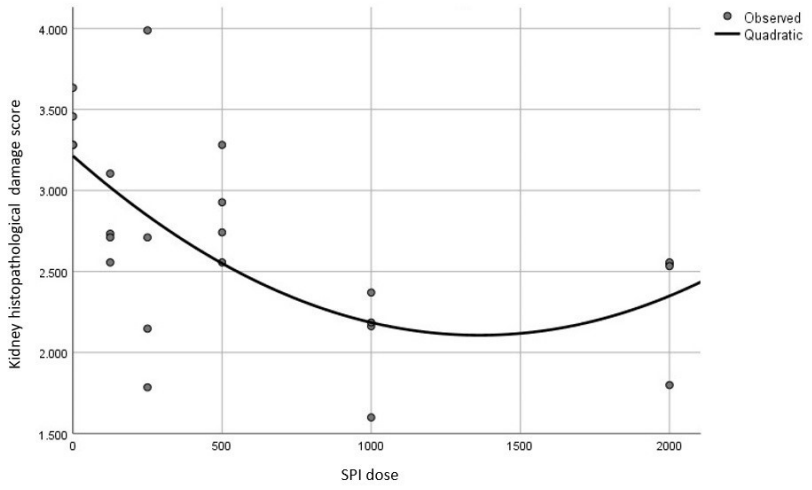


**Figure 2 The Regression Curve of SPI Dose and Kidney Histopathological Damage Score**

homeostasis. Renal tubular cells are unable to pump sodium ions out which causes the increase of sodium ions and intracellular osmotic pressure. This condition triggers the entry of water to the tubular lumen, so that the tubules get swelling and lose their brush-border.[18–20]

Necrosis is caused by loss of membrane integrity and leakage of cell contents related to diazinon toxic effect. Lipid peroxidation that occurs due to the increase of reactive oxygen species (ROS) induced by diazinon disrupts membrane permeability generating the impairment of kidney cell function which potentially triggers necrosis. The tubular cells undergoing necrosis will be shed and the cell components will be scattered in the lumen, hence there will be accumulation of cell debris in the lumen. The histopathological changes of nucleus during necrosis are shrinkage, irregular border, and color darkening.[18]

Tubular casts are formed when there is a large leakage of protein in the glomerular filtrate, so that the tubules are no longer able to reabsorb proteins entering the lumen. It causes excessive accumulation and deposition of protein in vesicles related to tubular dilatation.[18] In addition, oxidative stress in the kidney causes infiltration of immune cells through activation of proinflammatory cytokines and chemokines by ROS, demonstrated by the increase of neutrophil infiltration in kidney tissue.[19]

Kidney histopathological appearance in the treatment groups showed amelioration along with the increase of SPI dose. The result of Pearson correlation test showed moderate correlation with coefficient correlation -0.594. It indicates that amelioration of kidney histopathological damage is affected 59.4% by SPI, while 40.6% is affected by other factors including self-repair mechanism of the rat body in 7 days which may also be influenced by foods and environment. The result of correlation did not get a strong correlation. This is due to the flavonoid level in infusion is not as high as the flavonoid level in extract. The impact of SPI administration is accelerating the repair of kidney histopathological damage; the higher the SPI dose, the lower kidney histopathological damage score. It indicates that kidney histopathological improvement depends on the dose of SPI. Flavonoids neutralize the toxic effects of free radicals by transferring hydrogen ions and the molecules become more stable. This condition will reduce oxidative stress in the tissues and cellular damage.[21–23] Flavonoids also increase prostaglandin production resulting vasodilation and the increase of kidney perfusion. Furthermore, flavonoids promote BMDCs to be released from bone marrow into the peripheral blood and move into the damaged area after attracted by growth factors and inflammatory cytokines released from the injured area. It was discovered that engrafted BMDCs into the damaged cells plays an essential role in normal cell turnover to improve membrane integrity loss. In a previous study, quercetin induced bone marrow mesenchymal stem cells proliferation and osteogenic differentiation by increasing bone morphogenetic protein (BMP) signaling pathway activation and upregulates downstream genes expression such as *OPN, RUNX2*, and *OSX*.[12]

The administration of SPI exceeds the maximum effective dose is no longer effective to ameliorate diazinon-induced kidney damage. The kidney histopathological damage score at a dose of 2,000 mg/kgBW exceeds the damage score at a dose of 1,359 mg/kgBW. This is presumably because flavonoid contained in SPI above the maximum effective dose changes its properties from antioxidant to pro-oxidant so that it is unable to neutralize oxidative stress and the cell damage process continues.[24] The previous study stated that the ethyl acetate extract of shallot peel doses of 1,600 mg/kgBW. and 2,900 mg/kgBW for 14 days caused liver damage in mice including sinusoidal dilatation, tissue bleeding, and lymphocyte aggregation.[25]

It can be concluded that the SPI dose and the amelioration of kidney histopathological damage are moderately correlated; the higher the SPI dose, the lower the kidney histopathological damage. The maximum effective dose of SPI to ameliorate kidney histopathological damage in diazinon-induced Wistar rats is 1,359 mg/kgBW. Related to the limitations of this study, it is needed to measure the levels of flavonoids in SPI, analyze the structure of the kidney using quantitative stereology methods, and carry out kidney function test. In addition, it is also necessary to measure kidney malondialdehyde (MDA) as an indicator of oxidative stress.

## References

1. Wang D, Singhasemanon N, Goh KS. A Review of diazinon use, contamination in surface waters, and regulatory actions in california across water years 1992–2014. Environ Monit Assess. 2017;189(7):310. doi:10.1007/s10661-017-6026-z
2. Ardiwinata AN, Ginoga LN, Sulaeman E,

Harsanti ES. pesticide residue monitoring on agriculture in Indonesia. Jurnal Sumberdaya Lahan. 2018;12(2):133–44.
3. Al-Attar AM, Elnaggar MHR, Almalki EA. Protective effect of some plant oils on diazinon induced hepatorenal toxicity in male rats. Saudi J Biol Sci. 2017;24(6):1162–71. doi:https://doi.org/10.1016/j.sjbs.2016.10.013
4. Cakici O, Akat E. Effects of oral exposure to diazinon on mice liver and kidney tissues: biometric analyses of histopathologic changes. Anal Quant Cytopathol Histopahtol. 2013;35(1):7–16.
5. Wisudanti DD, Herdiana F, Qodar TS. diazinon toxicity to kidney and liver of wistar male rats in terms of biochemical and histopathological parameters. J Agromedicine Med Sci. 2019;5(2):112–7. doi:10.19184/ams.v5i2.11575
6. Pearson JN, Patel M. The role of oxidative stress in organophosphate and nerve agent toxicity. Ann N Y Acad Sci. 2016;1378(1):17–24. doi:https://doi.org/10.1111/nyas.13115
7. Bhandari SR, Yoon MK, Kwak JH. Contents of phytochemical constituents and antioxidant activity of 19 garlic (Allium sativum L.) Parental Lines Cultivars. Hortic Environ Biotechnol. 2014;55(2):138–47. doi:https://doi.org/10.1007/s13580-014-0155-x
8. Shi GQ, Yang J, Liu J, Liu SN, Song HX, Zhao WE, et al. Isolation of flavonoids from onion skin and their effects on K562 cell viability. Bangladesh J Pharmacol. 2016;11:18–25. doi:https://doi.org/10.3329/bjp.v11iS1.26419
9. Mardiah N, Mulyanto C, Amelia A, Lisnawati L, Anggraeni D, Rahmawanty D. Penentuan aktivitas antioksidan dari ekstrak kulit bawang merah dengan metode DPPH. Jurnal Pharmascience. 2017;4(2):147–54. doi:10.20527/jps.v4i2.5768
10. Viera VB, Piovesan N, Rodrigues JB, Mello R de O, Prestes RC, dos Santos RCV, et al. Extraction of phenolic compounds and evaluation of the antioxidant and antimicrobial capacity of red onion skin (Allium cepa L.). Int Food Res J. 2017;24(3):990.
11. Rahima SA, Dewi R, Rusmatika NS, Helianti D. Shallot (Allium cepa L.) skin ethanol extract neutralizes liver oxidative stress in diazinon-induced wistar rats. Qanun Medika. 2022;6(1):49–58.
12. Pang XG, Cong Y, Bao NR, Li YG, Zhao JN. Quercetin Stimulates Bone Marrow Mesenchymal Stem Cell Differentiation through an Estrogen Receptor-Mediated Pathway. Biomed Researcch International. 2018;2018:1–11. doi:https://doi.org/10.1155/2018/4178021
13. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. Sci World J. 2013;2013:1–16. doi:https://doi.org/10.1155/2013/162750
14. Universitas Indonesia. Acuan Sediaan Herbal, 2010. Indonesia: Katalog Perpustakaan Terpadu Poltekkes Kemenkes Jakarta II; 2010.
15. Kocoglu H, Ozturk H, Ozturk H, Yilmaz F, Gulcu N. Effect of dexmedetomidine on ischemia-reperfusion injury in rat kidney: a histopathologic study. Renal Failure. Ren Fail. 2009;31(1):70–4. doi:https://doi.org/10.1080/08860220802546487
16. Bleier L, Wittig I, Heide H, Steger M, Brandt U, Dröse S. generator-specific targets of mitochondrial reactive oxygen species. Free Radic Biol Med. 2015;78:1–10. doi:https://doi.org/10.1016/j.freeradbiomed.2014.10.511
17. Miranda CA, Guimarães AR de JS, Bizerra PFV, Mingatto FE. diazinon impairs bioenergetics and induces membrane permeability transition on mitochondria isolated from rat liver. J Toxicol Environ Heal-Part A Curr Issues. 2020;83(17-18):616–29. doi:https://doi.org/10.1080/15287394.2020.1805078
18. Kumar V, Abbas AK, Aster JC. Robbins Basic Pathology. 9th ed. Chicago: Elsevier Inc.; 2013.
19. Restuati M, Panggabean E. Pengaruh pemberian ekstrak etanol daun sisrak (annona muricata l.) terhadap gambaran histopatologi organ ginjal dan hati tikus putih (rattus norvegicus l.) dengan pemberian SRBC sebagai antigen. Seminar Nasional Biologi dan Pembelajarannya; 2014 Aug 23; Medan, Indonesia.
20. Sari WN, Saebani, Dhanardhono T. Pengaruh pemberian butylated hydroxytoluene (2,6-di-tert-butyl-4-methylphenol) per oral dosis bertingkat terhadap gambaran histopatologis hepar tikus wistar. Diponegoro Med J (Jurnal Kedokt Diponegoro). 2018;7(2):1154–65.
21. Tandi J, Wulandari A, Asrifa. Efek ekstrak etanol daun gendola merah (Basella alba L.) terhadap kadar kreatinin, ureum dan deskripsi histologis tubulus ginjal tikus putih jantan (rattus norvegicus) diabetes yang diinduksi streptozotocin. J Farmasi Galenika. 2017;3(2):93–102. doi:https://

doi.org/10.22487/j24428744.0.v0.i0.8813
22. Vásquez-Espinal A, Yañez O, Osorio2 E, Areche C, García-Beltrán O, Ruiz LM, et al. Theoretical study of the antioxidant activity of quercetin oxidation products. Front Chem. 2019;7(818):1–10. doi:https://doi.org/10.3389/fchem.2019.00818
23. Xu D, Hu MJ, Wang YQ, Cui YL. Antioxidant activities of quercetin and its complexes for medicinal application. Molecules. 2019;24(6):1123. doi:10.3390/molecules24061123
24. Mateus PG, Wolf VG, Borges MS, Ximenes VF. Quercetin: prooxidant effect and apoptosis in cancer. Studies in Natural Products Chemistry. 2018;58:265–88.
25. Dibal NI, Hyedima Garba S, Watson Jacks T. acute toxicity of quercetin from onion skin in mice. Pharmaceutical Biomed Res. 2021;6(4):269–76. doi:10.18502/pbr.v6i4.5113

RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v56.3107
Majalah Kedokteran Bandung. 2024;56(1):23–28

Majalah Kedokteran Bandung (MKB)

Received: November 24, 2022
Accepted: October 20, 2023
Available online: March 31, 2024

# Predictive Factors of Amputation for Post-Bypass Surgery on Vascular Trauma Patients

**Afila Reza Kusworo,[1] Heroe Soebroto,[2] Heri Suroto[3]**

[1]Medical Program in Faculty of Medicine, Airlangga University, Surabaya, Indonesia
[2]Department of Cardiothoracic and Vascular Surgery, Faculty of Medicine, Universitas Airlangga, Surabaya/Dr. Soetomo General Academic Hospital, Surabaya, Indonesia
[3]Department of Orthopaedic and Traumatology, Faculty of Medicine, Universitas Airlangga, Surabaya/ Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

**Abstract**

In Indonesia, most vascular trauma is linked to traffic collisions. According to the Statistics Indonesia (Badan Pusat Statistik, BPS), there were 116,411 accidents in 2019. Limb salvages become important in vascular trauma, which is performed through, among others, vascular bypass surgery. However, secondary amputation still occurs after vascular bypass. This study aimed to determine the predictors of secondary amputation after vascular bypass due to vascular trauma. This study used descriptive analysis of medical records and univariate analysis. Medical records of patients with vascular trauma underwent vascular bypass in Dr Soetomo Regional Hospital, Indonesia, from January 2018 to December 2020 were collected. Independent variables were age, MESS, time interval between the incident of trauma to the first incision of bypass surgery, penetrating and blunt injury, injured arterial segment, multiple injuries, and obesity. The dependent variable was secondary amputation. Fisher Exact Test was used to analyze the correlation between dependent and independent variables. Results showed a significant difference between patients with a MESS score of >7 and those with a MESS score of ≤7 (p=0.044), where more patients with a MESS score of >7 experienced secondary amputation. Other variables showed no significant difference (p>0.05). This study concluded that MESS could be used as a predictor of secondary amputation in vascular trauma after vascular bypass. Further studies using multivariate analysis and a larger sample need to be conducted to get further insights on this phenomenon.

**Keywords:** Bypass surgery, MESS, predictor, traffic accident, vascular trauma, vascular bypass surgery

## Introduction

Statistic reports about vehicle accidents in Indonesia increased from 2017 to 2019. The number increased from 104 327,00 in 2017, 109 215,00 in 2018, to 116 411,00 in 2019.[1] The statistics were vital because vehicle accidents are closely related to vascular trauma in Indonesia. Vascular trauma is a lesion in an artery or vein caused by blunt and penetrating mechanisms. Blunt trauma is identified as vehicle accidents or collisions where the patient is the driver, accidents in pedestrians, injuries to motorcycle or bicycle riders, falls, injuries due to explosions, or planned attacks on people. Penetrating trauma happens due to foreign objects penetrating the tissues. The common objects are high-energy weapons (military or hunting rifles), medium-energy weapons (handguns), and low-energy weapons (knives, hand-energized missiles).[2] Vascular trauma is an urgent condition that needs immediate and correct management to keep the tissue viable.[3]

The prognosis of vascular trauma is based on the types and anatomical region. Compression fracture usually has more severe tissue necrosis. A proximal tibia fracture will have a better prognosis if the repair is done quickly. One of the operative management is vascular bypass surgery. Even though the bypass surgery was done, the possibility of amputation is still high in some cases. The number of failed bypasses is higher in blunt trauma cases.[3] Amputation could be a choice if the bypass failed. The common reasons for failed bypass are thrombus, intima hyperplasia, or atherosclerotic plaque.[4] The high

**Corresponding Author:**
Heroe Soebroto,
Department of Cardiothoracic and Vascular Surgery, Faculty of Medicine, Universitas Airlangga, Surabaya/Dr. Soetomo General Academic Hospital, Surabaya, Indonesia
Email: afila.reza.kusworo-2019@fk.unair.ac.id

number of amputations after bypass surgery became a problem that needs to be overcome and predicted. In addition, the study about amputation predictors after bypass surgery is still limited. This study was conducted to find the predictor of amputation after bypass surgery. Thus, observing the prognostic factor could be used for clinical consideration in medical treatment.

## Methods

This descriptive-analytic and retrospective study was conducted at Dr. Soetomo General Hospital Surabaya. This study used a total sampling method to collect the data. Only data that met the criteria were included in this research. The inclusion criteria are patients with trauma vascular who underwent vascular bypass surgery, patients with complete medical records, and patients who underwent amputation after vascular bypass surgery in Dr. Soetomo General Hospital from January 1, 2018, until December 31, 2020.

The data were collected from medical records in Dr. Soetomo General Hospital. The medical records were collected from Medical Records Center and the Cardiothoracic and Vascular Surgery Department in Dr. Soetomo General Hospital. This study's independent variables mentioned were age, MESS, the time interval between the incident of trauma to first incision of bypass surgery, penetrating trauma, blunt trauma, injured arterial segment, multiple injuries (multiple injuries described as injuries other than vessels injury, such as nerve injuries, fractures, muscle ruptures), and obesity. The dependent variable is secondary amputation.

The data were analyzed with the Fisher Exact Test using IBM SPSS Statistics 23. The test was a univariate analysis with a significance level of 5%. The Research Health Ethic Committee (protocol number: 0951/110/4/IX/2021) approved the ethical clearance of Dr. Soetomo General Hospital Surabaya.

## Results

There were 17 data of medical records included in this study. All the bypass procedure was performed using native GSV. The procedures were done by a cardiothoracic and vascular surgeon as the operator and accompanied by the cardiothoracic and vascular surgeon residents. The mean age (years) of patients with vascular injury was 30.12±14.41, ranging from 3 years old to 58 years. MESS ranged from 3 to 10 with a mean of 7.94±2.22. The mean interval between the trauma incident and bypass surgery (minutes) was 1085.53±1700.69.

Table demonstrates each variable's correlation to secondary amputation using the Fisher exact test. The age was grouped into ≤35 and >35 years old. MESS is grouped into ≤7 and >7. The interval time between the first incision of bypass surgery and the time of trauma (hours). Two groups of trauma mechanisms are blunt and penetrating. The artery segment is grouped as non-end artery and end artery. Multiple traumas are divided into five groups based on the number of associated traumas. At the same time, obesity is grouped as absent or present. The result of the surgery was defined as present and absent of secondary amputation.

Based on the univariate analysis using the Fisher Exact Test, it was found that between two groups of age and secondary amputation has no significant difference with *p*-value=0.338. MESS is the only variable showing a significant difference with a p-value=0.044. It is found that a MESS higher than seven is likely to receive amputation after the bypass surgery. The time interval between trauma and bypass surgery had no significant difference to the result of secondary amputation, with a p-value=0.603. Neither blunt nor penetrating injuries were related to amputation after the bypass surgery, with a p-value=0.228. It was also found that two groups of artery segment and secondary amputation have no significant difference with p-value=0.537. Multiple traumas also did not significantly differ from secondary amputation, with p-value=0.090. Moreover, the last variable, the presence or absence of obesity, had no significant difference to amputation after the bypass surgery, with p-value=0.331.

## Discussion

Seventeen out of 32 medical reports met the criteria. The other 15 data could not be used due to lack of comprehensiveness in the medical records and or underwent primary amputation. Along with the small amount of data, it became a limitation for this study.

MESS has been known and used as a scoring system all around the world. Johansen first introduced it in 1990. Four variables included are skeletal or soft tissue injury, signs of limb

**Table 1 Univariate Analysis Using the Fisher Exact Test**

| Variable | Secondary Amputation | | p-value |
|---|---|---|---|
| | Present | Absent | |
| Age (years) | | | 0.338 |
| ≤35 | 6 | 6 | |
| >35 | 1 | 4 | |
| MESS[1*] | | | 0.044 |
| ≤7 | 5 | 0 | |
| >7 | 5 | 7 | |
| The interval time between first incision of bypass surgery and time of trauma (hours) | | | 0.603 |
| ≤6 | 3 | 1 | |
| >6 | 7 | 6 | |
| Trauma mechanism | | | 0.228 |
| Penetrating | 4 | 0 | |
| Blunt | 7 | 6 | |
| Artery segment | | | 0.537 |
| Non-end artery | 9 | 5 | |
| End artery | 1 | 2 | |
| Multiple trauma | | | 0.090 |
| ≥3 | 4 | 5 | |
| 0 | 2 | 0 | |
| 1 | 3 | 0 | |
| 2 | 1 | 0 | |
| POST-ORIF | 0 | 2 | |
| Obese | | | 0.331 |
| Present | 2 | 0 | |
| Absent | 8 | 7 | |

ischemia, shocks, and patient age. The higher the scores, the greater the chance of amputation. MESS analysis as trauma prognosis has been widely performed and shows a good correlation between amputation and high scores. A previous study found that the sensitivity and NPV of MESS was 100% which indicates that this scoring system could identify how likely a limb could be saved.[5] Another study also found a poor prognosis in trauma patients with a MESS score of more than 9.[6] The finding in this study indicated that MESS could also predict secondary amputation. It is shown as a significant difference of *P* value less than 0.005. A MESS score of more than 7 is higher in secondary amputation rate than patients with scores of ≤ 7**.**

Age is considered a dire prognosis of limb salvage.[7] Aging is closely related to changes in the human body. Vascular stiffening and vascular changes are part of the normal aging process. Moreover, other diseases such as diabetes and hypertension are higher in the elderly. Thus, older patients are susceptible to injury and acute ischemia.[8] Another study stated the relation between age over 40 and bad clinical outcomes.[6] In addition, other research on the Indonesian population found that age above 35 has a higher prevalence of hypertension than the younger group.[9] According to the research conducted by Maharani, adults aged 40 years and above have a higher risk of cardiovascular disease in the next 10 years of their life.[10] Based on previous research, the age grouping in this article was ≤35 and >35 years old. However, this study found no significant difference between the two age groups (p=0.63). In addition, another study also showed no significant difference between the older and younger age groups.[11] Furthermore, we did not find any comorbidities such as diabetes and hypertension in 17 patients who participated in this study, while age is closely related to such diseases.

The time of tissue ischemia, often called the golden period, is no more than 6 hours. A study

on 35 patients with grade III C fractures showed an association. The delay in revascularization intervention significantly indicates a higher incidence of secondary amputation.[12] The maximum time limit for limb ischemia to be salvaged with a good prognosis is 6 hours.[13] Hunter conducted a study on 108 patients undergoing infrainguinal bypass surgery, where the time interval between trauma and surgery did not significantly differ in the outcome.[6] This study used the time interval between the trauma incidence and the bypass surgical treatment. The results are the same as the research conducted by Hunter in that a time interval of more than 6 hours does not indicate a significantly poor prognosis. However, this result is different from the existing textbooks. Previous studies regarding the prognosis of amputation after tissue ischemia of more than 12 hours were strongly associated with the presence of collateral circulation. More collateral vascularization can help maintain tissue viability even after arterial trauma beyond the golden period. However, sensory and motor function in prolonged ischemia is poor.[14] Data from medical records showed that one patient underwent secondary amputation even though the time interval between the incidence of trauma and bypass surgery is less than 6 hours. However, despite the time being still in the golden period, the MESS is 10, which might explain the presence of secondary amputation. Other seven patients with a time interval of more than 6 hours without secondary amputation have a MESS of less than 10. Other findings from medical records are that two patients with the same MESS (MESS=9) and the same time interval of more than 6 hours have different outcomes, amputated and salvaged. After observing the medical records, they have different artery and trauma lesions. The patient with a secondary amputation had a tibial shaft fracture and, an open distal femur fracture with anterior and posterior tibial artery lesions. In comparison, the patient without amputation had ⅓ distal of the posterior tibial artery and ⅓ distal of the tibia-fibula open fracture, open lesion on a knee, and knee dislocation. This finding could be explained by a previous study that found distal femur or knee fracture has a high risk of bad prognosis for the distal limb affected. Moreover, an open fracture accompanied by a lesion of the anterior and posterior tibial artery in the trifurcation level led to the worst prognosis of limb salvage.[15]

Identifying trauma mechanisms could predict vascular trauma. Gunshot injury is more likely to result in more complex vascular trauma and a higher amputation rate than stabbed injury. In addition, blunt trauma usually causes vascular injury.[1316] Nevertheless, another study stated that blunt trauma does not directly cause vascular trauma. The limb salvage could not be reached due to failed revascularization, injury to soft tissue, and osteomyelitis.[17] Based on a univariate study conducted by Hunter, blunt trauma significantly predicts worse outcomes such as death, primary amputation, secondary amputation, and failed bypass surgery.[6] In contrast to previous studies, this univariate study results in no significant difference between blunt trauma and the incidence of secondary amputation. This study has the same result as a previous study about amputation in injury to the popliteal artery, in which blunt and penetrating trauma did not significantly differ from the worst outcome of amputation.[18]

In addition to the above variables, this study examined whether trauma affects specific arterial segments and risk factors for secondary amputation. Trauma to the arterial segment did not have a significant difference that could be used to predict the presence of secondary amputation. Three non-end artery injuries that underwent secondary amputation had a MESS score of 10, and the other had a MESS score of 9. One of the patients with a MESS score of 9 had compartment syndrome. The presence of amputation secondary to an injured artery that may still have collateral circulation may result from a high MESS score.

The reason obesity could be considered as a predictive factor is because it is closely related to dyslipidemia. Atherosclerotic plaque formation and the emergence of cardiovascular disease are often found in patients with obesity.[19] Obesity was found in two patients and both of them achieved limb salvage. As in these cases, the first obese patient who underwent bypass had an ischemic time under 6 hours (still in the golden period) and already received emergency treatment in RSUD Jombang before being admitted to Dr. Soetomo General Hospital. Along with that, as stated in the primary survey, the patient arrived at Dr. Soetomo with GCS 456 and CRT in less than 2 seconds. Another obese patient who achieved limb salvage also came after receiving emergency aid in Babat Hospital and as in the primary survey, this patient came with CRT in less than 2 seconds. However, the finding in this study is no significant differences between the two groups. A prior study observed the relationship between obesity and amputation-free survival conducted by Sabbagh and found no association among

those variables.[20]

This study describes fractures, muscle ruptures, and nerve injuries as multiple injuries. No significant differences were found after Fisher's exact test of 0, 1, 2, and ≥3 associated injuries or post-ORIF. Another study by Song stated that there is a correlation between associated injuries to failed limb salvage.[1211] Like the previous study, multiple injuries could not be an amputation predictor post-bypass surgery.

The significant difference in MESS is 0.044 ($p<0.05$), so high MESS could be an independent predictor for amputation after bypass surgery. Furthermore, it is crucial to consider the proper treatment, further prevention, and precaution for secondary amputation because each treatment would affect the patient's quality of life.

This study concluded that in the Indonesian population, specifically, patients admitted to Dr. Soetomo General Hospital, MESS >7 is an independent predictor of secondary amputation in patients with vascular trauma who underwent vascular bypass surgery. The higher MESS showed a lousy prognosis for secondary amputation. Age, the time interval between trauma and bypass surgery, penetrating trauma, blunt trauma, injured arterial segment, multiple injuries, and obesity are not predictors of secondary amputation in this study. However, the limited sample of this study needs to be considered.

## References


1. BPS. Badan Pusat Statistik. Jumlah Kecelakaan, Korban Mati, Luka Berat, Luka Ringan, dan Kerugian Materi, 2020. Statistik Indonesia 2020. Jakarta: Statistical Yearbook Indonesia; 2020.
2. Huber GH, Manna B. Vascular Extremity Trauma. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2022.
3. Suhardi. Tatalaksana Trauma Vascular. In: Aceh Surgery Update 2; 2017.
4. Gaudino M, Antoniades C, Benedetto U, Deb S, Franco DA, Giammarco DG, et al. Mechanisms, consequences, and prevention of coronary graft failure. Circulation. 2017;136(18):1749–64. doi:10.1161/CIRCULATIONAHA.117.027597
5. abbani S, Azam Haseen M, Ali Rizwi A, Hanif Beg M. Can mangled extremity scoring system (MESS) solve the mess of vascular trauma. 2020;7(1):22–8 DOI: 10.4103/ijves.ijves_44_19
6. Ray HM, Sandhu HK, Meyer DE, Miller CC, Vowels TJ, Afifi RO, et al. Predictors of poor outcome in infrainguinal bypass for trauma. In: J Vascular Surgery. Mosby Inc. 2019;70(6):1816–22.
7. Verwer MC, Wijnand JGJ, Teraa M, Verhaar MC, de Borst GJ. Long term survival and limb salvage in patients with non-revascularisable chronic limb threatening ischaemia. ESVS. 2021;62(2):225–32. doi:10.1016/j.ejvs.2021.04.003
8. Vatner SF, Zhang J, Vyzas C, Mishra K, Graham RM, Vatner DE. Vascular stiffness in aging and disease. Front Physiol. 2021;12:762437. doi:10.3389/fphys.2021.762437
9. Tirtasari S, Kodim N. Prevalensi Dan karakteristik hipertensi pada usia dewasa muda di Indonesia. Tarumanagara Medical Journal. 2019;1(2):395–402.
10. Maharani A, Sujarwoto, Praveen D, Oceandy D, Tampubolon G, Patel A. Cardiovascular disease risk factor prevalence and estimated 10-year cardiovascular risk scores in Indonesia: the SMARThealth extend study. PLoS One. 2019;14(4):e0215219. doi:10.1371/journal.pone.0215219
11. Al-Zoubi NA, Shatnawi NJ, Khader Y, Heis M, Aleshawi AJ. predictive factors for failure of limb salvage in blunt leg trauma associated with vascular injuries. J Emerg Trauma Shock. 2021;14(2):80–5. doi:10.4103/JETS.JETS_37_20
12. Song W, Zhou D, Dong J. Predictors of secondary amputation in patients with grade IIIC lower limb injuries. Medicine (United States). 2017;96(22):e7068. doi:10.1097/MD.0000000000007068
13. Puruhito. Penyakit Arteria. In: Puruhito, editor. Buku Ajar Primer : Ilmu Bedah Toraks, Kardiak, Dan Vaskular. 1st ed. Surabaya: AUP; 2013. p. 347-352
14. Yu L, Deng L, Zhu S, Deng K, Yu G, Zhu C, et al. Limb-salvage outcomes of arterial repair beyond time limit at different lower-extremity injury sites. Medical Science Monitor. 2020;27:e927652. doi:10.12659/MSM.927652
15. Soucacos P, Kokkalis. Fractures with Arterial Injury. In: Bentley G, editor. European Surgical Orthopaedics and Traumatology. 1st ed. Heidelberg: Springer Berlin; 2014. p. 183.
16. Ramdass M, Harnarayan P. A decade of major vascular trauma: Lessons learned from gang and civilian warfare. Ann R Coll Surg Engl. 2017;99(1):70–5. doi:10.1308/rcsann.2016.0296

17. Kim J, Jeon YS, Cho SG, Hong KC, Park KM. Risk factors of amputation in lower extremity trauma with combined femoropopliteal arterial injury. Vasc Specialist Int. 2019;35(1):16–21. doi:10.5758/vsi.2019.35.1.16
18. 18. Ramdass MJ, Muddeen A, Harnarayan P, Spence R, Milne D. Risk factors associated with amputation in civilian popliteal artery trauma. Injury. 2018;49(6):1188–92. doi:10.1016/j.injury.2018.04.028
19. Howard B V, Ruotolo G, Robbins DC. Obesity and dyslipidemia. Endocrinol Metab Clin North Am. 2003;32(4):855–67. doi:10.1016/s0889-8529(03)00073-2
20. Sabbagh C, Nickinson A, Cullen S, Patel B, Dubkova S, Gray L, et al. The relationship between obesity and amputation-free survival in patients undergoing lower-limb revascularisation for chronic limb-threatening ischaemia: a retrospective cohort study. Ann Vasc Surg. 2022;78:288–94. doi:10.1016/j.avsg.2021.06.022

RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v56.3107
Majalah Kedokteran Bandung. 2024;56(1):23–28

Majalah Kedokteran Bandung (MKB)

Received: November 24, 2022
Accepted: October 20, 2023
Available online: March 31, 2024

# Predictive Factors of Amputation for Post-Bypass Surgery on Vascular Trauma Patients

**Afila Reza Kusworo,[1] Heroe Soebroto,[2] Heri Suroto[3]**

[1]Medical Program in Faculty of Medicine, Airlangga University, Surabaya, Indonesia
[2]Department of Cardiothoracic and Vascular Surgery, Faculty of Medicine, Universitas Airlangga, Surabaya/Dr. Soetomo General Academic Hospital, Surabaya, Indonesia
[3]Department of Orthopaedic and Traumatology, Faculty of Medicine, Universitas Airlangga, Surabaya/ Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

**Abstract**

In Indonesia, most vascular trauma is linked to traffic collisions. According to the Statistics Indonesia (Badan Pusat Statistik, BPS), there were 116,411 accidents in 2019. Limb salvages become important in vascular trauma, which is performed through, among others, vascular bypass surgery. However, secondary amputation still occurs after vascular bypass. This study aimed to determine the predictors of secondary amputation after vascular bypass due to vascular trauma. This study used descriptive analysis of medical records and univariate analysis. Medical records of patients with vascular trauma underwent vascular bypass in Dr Soetomo Regional Hospital, Indonesia, from January 2018 to December 2020 were collected. Independent variables were age, MESS, time interval between the incident of trauma to the first incision of bypass surgery, penetrating and blunt injury, injured arterial segment, multiple injuries, and obesity. The dependent variable was secondary amputation. Fisher Exact Test was used to analyze the correlation between dependent and independent variables. Results showed a significant difference between patients with a MESS score of >7 and those with a MESS score of ≤7 (p=0.044), where more patients with a MESS score of >7 experienced secondary amputation. Other variables showed no significant difference (p>0.05). This study concluded that MESS could be used as a predictor of secondary amputation in vascular trauma after vascular bypass. Further studies using multivariate analysis and a larger sample need to be conducted to get further insights on this phenomenon.

**Keywords:** Bypass surgery, MESS, predictor, traffic accident, vascular trauma, vascular bypass surgery

## Introduction

Statistic reports about vehicle accidents in Indonesia increased from 2017 to 2019. The number increased from 104 327,00 in 2017, 109 215,00 in 2018, to 116 411,00 in 2019.[1] The statistics were vital because vehicle accidents are closely related to vascular trauma in Indonesia. Vascular trauma is a lesion in an artery or vein caused by blunt and penetrating mechanisms. Blunt trauma is identified as vehicle accidents or collisions where the patient is the driver, accidents in pedestrians, injuries to motorcycle or bicycle riders, falls, injuries due to explosions, or planned attacks on people. Penetrating trauma happens due to foreign objects penetrating the tissues. The common objects are high-energy weapons (military or hunting rifles), medium-energy weapons (handguns), and low-energy weapons (knives, hand-energized missiles).[2] Vascular trauma is an urgent condition that needs immediate and correct management to keep the tissue viable.[3]

The prognosis of vascular trauma is based on the types and anatomical region. Compression fracture usually has more severe tissue necrosis. A proximal tibia fracture will have a better prognosis if the repair is done quickly. One of the operative management is vascular bypass surgery. Even though the bypass surgery was done, the possibility of amputation is still high in some cases. The number of failed bypasses is higher in blunt trauma cases.[3] Amputation could be a choice if the bypass failed. The common reasons for failed bypass are thrombus, intima hyperplasia, or atherosclerotic plaque.[4] The high

**Corresponding Author:**
Heroe Soebroto,
Department of Cardiothoracic and Vascular Surgery, Faculty of Medicine, Universitas Airlangga, Surabaya/Dr. Soetomo General Academic Hospital, Surabaya, Indonesia
Email: afila.reza.kusworo-2019@fk.unair.ac.id

number of amputations after bypass surgery became a problem that needs to be overcome and predicted. In addition, the study about amputation predictors after bypass surgery is still limited. This study was conducted to find the predictor of amputation after bypass surgery. Thus, observing the prognostic factor could be used for clinical consideration in medical treatment.

## Methods

This descriptive-analytic and retrospective study was conducted at Dr. Soetomo General Hospital Surabaya. This study used a total sampling method to collect the data. Only data that met the criteria were included in this research. The inclusion criteria are patients with trauma vascular who underwent vascular bypass surgery, patients with complete medical records, and patients who underwent amputation after vascular bypass surgery in Dr. Soetomo General Hospital from January 1, 2018, until December 31, 2020.

The data were collected from medical records in Dr. Soetomo General Hospital. The medical records were collected from Medical Records Center and the Cardiothoracic and Vascular Surgery Department in Dr. Soetomo General Hospital. This study's independent variables mentioned were age, MESS, the time interval between the incident of trauma to first incision of bypass surgery, penetrating trauma, blunt trauma, injured arterial segment, multiple injuries (multiple injuries described as injuries other than vessels injury, such as nerve injuries, fractures, muscle ruptures), and obesity. The dependent variable is secondary amputation.

The data were analyzed with the Fisher Exact Test using IBM SPSS Statistics 23. The test was a univariate analysis with a significance level of 5%. The Research Health Ethic Committee (protocol number: 0951/110/4/IX/2021) approved the ethical clearance of Dr. Soetomo General Hospital Surabaya.

## Results

There were 17 data of medical records included in this study. All the bypass procedure was performed using native GSV. The procedures were done by a cardiothoracic and vascular surgeon as the operator and accompanied by the cardiothoracic and vascular surgeon residents. The mean age (years) of patients with vascular injury was 30.12±14.41, ranging from 3 years old to 58 years. MESS ranged from 3 to 10 with a mean of 7.94±2.22. The mean interval between the trauma incident and bypass surgery (minutes) was 1085.53±1700.69.

Table demonstrates each variable's correlation to secondary amputation using the Fisher exact test. The age was grouped into ≤35 and >35 years old. MESS is grouped into ≤7 and >7. The interval time between the first incision of bypass surgery and the time of trauma (hours). Two groups of trauma mechanisms are blunt and penetrating. The artery segment is grouped as non-end artery and end artery. Multiple traumas are divided into five groups based on the number of associated traumas. At the same time, obesity is grouped as absent or present. The result of the surgery was defined as present and absent of secondary amputation.

Based on the univariate analysis using the Fisher Exact Test, it was found that between two groups of age and secondary amputation has no significant difference with *p*-value=0.338. MESS is the only variable showing a significant difference with a p-value=0.044. It is found that a MESS higher than seven is likely to receive amputation after the bypass surgery. The time interval between trauma and bypass surgery had no significant difference to the result of secondary amputation, with a p-value=0.603. Neither blunt nor penetrating injuries were related to amputation after the bypass surgery, with a p-value=0.228. It was also found that two groups of artery segment and secondary amputation have no significant difference with p-value=0.537. Multiple traumas also did not significantly differ from secondary amputation, with p-value=0.090. Moreover, the last variable, the presence or absence of obesity, had no significant difference to amputation after the bypass surgery, with p-value=0.331.

## Discussion

Seventeen out of 32 medical reports met the criteria. The other 15 data could not be used due to lack of comprehensiveness in the medical records and or underwent primary amputation. Along with the small amount of data, it became a limitation for this study.

MESS has been known and used as a scoring system all around the world. Johansen first introduced it in 1990. Four variables included are skeletal or soft tissue injury, signs of limb

**Table 1 Univariate Analysis Using the Fisher Exact Test**

| Variable | Secondary Amputation | | p-value |
|---|---|---|---|
| | Present | Absent | |
| Age (years) | | | 0.338 |
| ≤35 | 6 | 6 | |
| >35 | 1 | 4 | |
| MESS[1*] | | | 0.044 |
| ≤7 | 5 | 0 | |
| >7 | 5 | 7 | |
| The interval time between first incision of bypass surgery and time of trauma (hours) | | | 0.603 |
| ≤6 | 3 | 1 | |
| >6 | 7 | 6 | |
| Trauma mechanism | | | 0.228 |
| Penetrating | 4 | 0 | |
| Blunt | 7 | 6 | |
| Artery segment | | | 0.537 |
| Non-end artery | 9 | 5 | |
| End artery | 1 | 2 | |
| Multiple trauma | | | 0.090 |
| ≥3 | 4 | 5 | |
| 0 | 2 | 0 | |
| 1 | 3 | 0 | |
| 2 | 1 | 0 | |
| POST-ORIF | 0 | 2 | |
| Obese | | | 0.331 |
| Present | 2 | 0 | |
| Absent | 8 | 7 | |

ischemia, shocks, and patient age. The higher the scores, the greater the chance of amputation. MESS analysis as trauma prognosis has been widely performed and shows a good correlation between amputation and high scores. A previous study found that the sensitivity and NPV of MESS was 100% which indicates that this scoring system could identify how likely a limb could be saved.[5] Another study also found a poor prognosis in trauma patients with a MESS score of more than 9.[6] The finding in this study indicated that MESS could also predict secondary amputation. It is shown as a significant difference of *P* value less than 0.005. A MESS score of more than 7 is higher in secondary amputation rate than patients with scores of ≤ 7**.**

Age is considered a dire prognosis of limb salvage.[7] Aging is closely related to changes in the human body. Vascular stiffening and vascular changes are part of the normal aging process. Moreover, other diseases such as diabetes and hypertension are higher in the elderly. Thus, older patients are susceptible to injury and acute ischemia.[8] Another study stated the relation between age over 40 and bad clinical outcomes.[6] In addition, other research on the Indonesian population found that age above 35 has a higher prevalence of hypertension than the younger group.[9] According to the research conducted by Maharani, adults aged 40 years and above have a higher risk of cardiovascular disease in the next 10 years of their life.[10] Based on previous research, the age grouping in this article was ≤35 and >35 years old. However, this study found no significant difference between the two age groups (p=0.63). In addition, another study also showed no significant difference between the older and younger age groups.[11] Furthermore, we did not find any comorbidities such as diabetes and hypertension in 17 patients who participated in this study, while age is closely related to such diseases.

The time of tissue ischemia, often called the golden period, is no more than 6 hours. A study

on 35 patients with grade III C fractures showed an association. The delay in revascularization intervention significantly indicates a higher incidence of secondary amputation.[12] The maximum time limit for limb ischemia to be salvaged with a good prognosis is 6 hours.[13] Hunter conducted a study on 108 patients undergoing infrainguinal bypass surgery, where the time interval between trauma and surgery did not significantly differ in the outcome.[6] This study used the time interval between the trauma incidence and the bypass surgical treatment. The results are the same as the research conducted by Hunter in that a time interval of more than 6 hours does not indicate a significantly poor prognosis. However, this result is different from the existing textbooks. Previous studies regarding the prognosis of amputation after tissue ischemia of more than 12 hours were strongly associated with the presence of collateral circulation. More collateral vascularization can help maintain tissue viability even after arterial trauma beyond the golden period. However, sensory and motor function in prolonged ischemia is poor.[14] Data from medical records showed that one patient underwent secondary amputation even though the time interval between the incidence of trauma and bypass surgery is less than 6 hours. However, despite the time being still in the golden period, the MESS is 10, which might explain the presence of secondary amputation. Other seven patients with a time interval of more than 6 hours without secondary amputation have a MESS of less than 10. Other findings from medical records are that two patients with the same MESS (MESS=9) and the same time interval of more than 6 hours have different outcomes, amputated and salvaged. After observing the medical records, they have different artery and trauma lesions. The patient with a secondary amputation had a tibial shaft fracture and, an open distal femur fracture with anterior and posterior tibial artery lesions. In comparison, the patient without amputation had ⅓ distal of the posterior tibial artery and ⅓ distal of the tibia-fibula open fracture, open lesion on a knee, and knee dislocation. This finding could be explained by a previous study that found distal femur or knee fracture has a high risk of bad prognosis for the distal limb affected. Moreover, an open fracture accompanied by a lesion of the anterior and posterior tibial artery in the trifurcation level led to the worst prognosis of limb salvage.[15]

Identifying trauma mechanisms could predict vascular trauma. Gunshot injury is more likely to result in more complex vascular trauma and a higher amputation rate than stabbed injury. In addition, blunt trauma usually causes vascular injury.[1316] Nevertheless, another study stated that blunt trauma does not directly cause vascular trauma. The limb salvage could not be reached due to failed revascularization, injury to soft tissue, and osteomyelitis.[17] Based on a univariate study conducted by Hunter, blunt trauma significantly predicts worse outcomes such as death, primary amputation, secondary amputation, and failed bypass surgery.[6] In contrast to previous studies, this univariate study results in no significant difference between blunt trauma and the incidence of secondary amputation. This study has the same result as a previous study about amputation in injury to the popliteal artery, in which blunt and penetrating trauma did not significantly differ from the worst outcome of amputation.[18]

In addition to the above variables, this study examined whether trauma affects specific arterial segments and risk factors for secondary amputation. Trauma to the arterial segment did not have a significant difference that could be used to predict the presence of secondary amputation. Three non-end artery injuries that underwent secondary amputation had a MESS score of 10, and the other had a MESS score of 9. One of the patients with a MESS score of 9 had compartment syndrome. The presence of amputation secondary to an injured artery that may still have collateral circulation may result from a high MESS score.

The reason obesity could be considered as a predictive factor is because it is closely related to dyslipidemia. Atherosclerotic plaque formation and the emergence of cardiovascular disease are often found in patients with obesity.[19] Obesity was found in two patients and both of them achieved limb salvage. As in these cases, the first obese patient who underwent bypass had an ischemic time under 6 hours (still in the golden period) and already received emergency treatment in RSUD Jombang before being admitted to Dr. Soetomo General Hospital. Along with that, as stated in the primary survey, the patient arrived at Dr. Soetomo with GCS 456 and CRT in less than 2 seconds. Another obese patient who achieved limb salvage also came after receiving emergency aid in Babat Hospital and as in the primary survey, this patient came with CRT in less than 2 seconds. However, the finding in this study is no significant differences between the two groups. A prior study observed the relationship between obesity and amputation-free survival conducted by Sabbagh and found no association among

those variables.[20]

This study describes fractures, muscle ruptures, and nerve injuries as multiple injuries. No significant differences were found after Fisher's exact test of 0, 1, 2, and ≥3 associated injuries or post-ORIF. Another study by Song stated that there is a correlation between associated injuries to failed limb salvage.[1211] Like the previous study, multiple injuries could not be an amputation predictor post-bypass surgery.

The significant difference in MESS is 0.044 ($p<0.05$), so high MESS could be an independent predictor for amputation after bypass surgery. Furthermore, it is crucial to consider the proper treatment, further prevention, and precaution for secondary amputation because each treatment would affect the patient's quality of life.

This study concluded that in the Indonesian population, specifically, patients admitted to Dr. Soetomo General Hospital, MESS >7 is an independent predictor of secondary amputation in patients with vascular trauma who underwent vascular bypass surgery. The higher MESS showed a lousy prognosis for secondary amputation. Age, the time interval between trauma and bypass surgery, penetrating trauma, blunt trauma, injured arterial segment, multiple injuries, and obesity are not predictors of secondary amputation in this study. However, the limited sample of this study needs to be considered.

## References


1. BPS. Badan Pusat Statistik. Jumlah Kecelakaan, Korban Mati, Luka Berat, Luka Ringan, dan Kerugian Materi, 2020. Statistik Indonesia 2020. Jakarta: Statistical Yearbook Indonesia; 2020.
2. Huber GH, Manna B. Vascular Extremity Trauma. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2022.
3. Suhardi. Tatalaksana Trauma Vascular. In: Aceh Surgery Update 2; 2017.
4. Gaudino M, Antoniades C, Benedetto U, Deb S, Franco DA, Giammarco DG, et al. Mechanisms, consequences, and prevention of coronary graft failure. Circulation. 2017;136(18):1749–64. doi:10.1161/CIRCULATIONAHA.117.027597
5. abbani S, Azam Haseen M, Ali Rizwi A, Hanif Beg M. Can mangled extremity scoring system (MESS) solve the mess of vascular trauma. 2020;7(1):22–8 DOI: 10.4103/ijves.ijves_44_19
6. Ray HM, Sandhu HK, Meyer DE, Miller CC, Vowels TJ, Afifi RO, et al. Predictors of poor outcome in infrainguinal bypass for trauma. In: J Vascular Surgery. Mosby Inc. 2019;70(6):1816–22.
7. Verwer MC, Wijnand JGJ, Teraa M, Verhaar MC, de Borst GJ. Long term survival and limb salvage in patients with non-revascularisable chronic limb threatening ischaemia. ESVS. 2021;62(2):225–32. doi:10.1016/j.ejvs.2021.04.003
8. Vatner SF, Zhang J, Vyzas C, Mishra K, Graham RM, Vatner DE. Vascular stiffness in aging and disease. Front Physiol. 2021;12:762437. doi:10.3389/fphys.2021.762437
9. Tirtasari S, Kodim N. Prevalensi Dan karakteristik hipertensi pada usia dewasa muda di Indonesia. Tarumanagara Medical Journal. 2019;1(2):395–402.
10. Maharani A, Sujarwoto, Praveen D, Oceandy D, Tampubolon G, Patel A. Cardiovascular disease risk factor prevalence and estimated 10-year cardiovascular risk scores in Indonesia: the SMARThealth extend study. PLoS One. 2019;14(4):e0215219. doi:10.1371/journal.pone.0215219
11. Al-Zoubi NA, Shatnawi NJ, Khader Y, Heis M, Aleshawi AJ. predictive factors for failure of limb salvage in blunt leg trauma associated with vascular injuries. J Emerg Trauma Shock. 2021;14(2):80–5. doi:10.4103/JETS.JETS_37_20
12. Song W, Zhou D, Dong J. Predictors of secondary amputation in patients with grade IIIC lower limb injuries. Medicine (United States). 2017;96(22):e7068. doi:10.1097/MD.0000000000007068
13. Puruhito. Penyakit Arteria. In: Puruhito, editor. Buku Ajar Primer : Ilmu Bedah Toraks, Kardiak, Dan Vaskular. 1st ed. Surabaya: AUP; 2013. p. 347-352
14. Yu L, Deng L, Zhu S, Deng K, Yu G, Zhu C, et al. Limb-salvage outcomes of arterial repair beyond time limit at different lower-extremity injury sites. Medical Science Monitor. 2020;27:e927652. doi:10.12659/MSM.927652
15. Soucacos P, Kokkalis. Fractures with Arterial Injury. In: Bentley G, editor. European Surgical Orthopaedics and Traumatology. 1st ed. Heidelberg: Springer Berlin; 2014. p. 183.
16. Ramdass M, Harnarayan P. A decade of major vascular trauma: Lessons learned from gang and civilian warfare. Ann R Coll Surg Engl. 2017;99(1):70–5. doi:10.1308/rcsann.2016.0296

17. Kim J, Jeon YS, Cho SG, Hong KC, Park KM. Risk factors of amputation in lower extremity trauma with combined femoropopliteal arterial injury. Vasc Specialist Int. 2019;35(1):16–21. doi:10.5758/vsi.2019.35.1.16
18. 18. Ramdass MJ, Muddeen A, Harnarayan P, Spence R, Milne D. Risk factors associated with amputation in civilian popliteal artery trauma. Injury. 2018;49(6):1188–92. doi:10.1016/j.injury.2018.04.028
19. Howard B V, Ruotolo G, Robbins DC. Obesity and dyslipidemia. Endocrinol Metab Clin North Am. 2003;32(4):855–67. doi:10.1016/s0889-8529(03)00073-2
20. Sabbagh C, Nickinson A, Cullen S, Patel B, Dubkova S, Gray L, et al. The relationship between obesity and amputation-free survival in patients undergoing lower-limb revascularisation for chronic limb-threatening ischaemia: a retrospective cohort study. Ann Vasc Surg. 2022;78:288–94. doi:10.1016/j.avsg.2021.06.022

RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v56.3346
Majalah Kedokteran Bandung. 2024;56(1):29–34

Majalah Kedokteran Bandung (MKB)

Received: May 9, 2023
Accepted: October 22, 2023
Available online: March 31, 2024

# Association Between Screen Time, Vitamin A Consumption Behavior, and Computer Vision Syndrome in Preclinical Medical Students

**Harka Prasetya,[1,2] Atik Rahmawati,[1,2] Aisyah Putri Andira,[3] Suparmi[4]**
[1]Department of Ophthalmology, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang, Indonesia
[2]Sultan Agung Eye Center, Rumah Sakit Islam Sultan Agung, Semarang, Indonesia
[3]Faculty of Medicine, Universitas Islam Sultan Agung, Semarang, Indonesia
[4]Department of Biology, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang, Indonesia

**Abstract**

Computer Visual Syndrome (CVS) refers to visual disturbances induced by electronic media such as computers, laptops, tablets, and smartphones. This research aimed to determine the association between screen time, vitamin A consumption behavior, and CVS symptoms among preclinical medical students. Ninety-five students from a single class at the Faculty of Medicine, Universitas Islam Sultan Agung, Indonesia, participated in this cross-sectional study. Data were collected using an online survey platform in February 2022. The screen time, vitamin A consumption behavior, and CVS were measured using a validated questionnaire. The average daily screen time of the respondents was more than 10 h, with the majority having a moderate vitamin A consumption patterns (66.3%) and experienced CVS (63.2%). The most prevalent CVS symptom identified among these respondents was eye fatigue (62.1%), while double vision and inflamed or watery eyes were the least reported CVS symptoms (9.5% and 8.4%, respectively). The Chi-Square test revealed that there was no correlation between screen time and CVS ($p>0.05$), but there was a significant correlation between vitamin A consumption and CVS ($p<0.05$). In conclusion, CVS is observed among preclinical medical students due to increased screen time and decreased vitamin A consumption. Therefore, there is a need to develop strategies to manage screen time and vitamin A consumption among students at risk for developing a CVS during their academic careers.

**Keywords:** Computer vision syndrome (CVS), consumption behavior, screen time, vitamin A

## Introduction

Today, millions of medical students use computers (including desktops, laptops, and tablets) and other electronic displays (such as smartphones and electronic reading devices) daily for both educational and recreational purposes, both on campus and at home. Computer vision syndrome (CVS) has been linked to screen time, the amount of time spent on electronic devices or in front of a screen per day.[1] Prolonged screen time without regular breaks was associated with an increased risk of CVS.[2] CVS symptoms include impaired vision, visual fatigue (VF) or discomfort, diplopia, dry eye disease, redness, eye strain, irritation, headache, and shoulder, neck, and back pain.[3] Although the visual effects of computer use on adults have been studied, few studies have examined this issue in preclinical medical students.

Although the COVID-19 pandemic has not been an issue, the prevalence of CVS and the widespread use of electronic devices should heighten awareness. Screen time induces tear film instability and oxidative stress indices in tears and on the eye's surface, according to a cross-sectional study conducted at JSS Medical College in Mysuru. The average daily screen time was 5.13 h.[4] A study of 1884 adolescents in Singapore found a correlation between increased smartphone use and physical health issues, including musculoskeletal and visual symptoms.[5] Increased screen time during the pandemic compared to before the pandemic resulted in ocular health issues, such as eye strain, in 50.6% of online learning students.[6] A cross-sectional study conducted in China revealed that CVS had

**Corresponding Author:**
Suparmi dan Harka Prasetya,
Jl. Raya Kaligawe KM 4 Semarang, Indonesia
Email: suparmi@unissula.ac.id, harkaprasetya@unissula.ac.id

a moderately positive correlation with screen time among Bachelor of Medicine and Bachelor of Surgery (MBBS) students with the most significant frequency of digital screen time (7–9 h/week).[7]

In addition, CVS can be alleviated by changes in dietary habits, which result in unhealthy food selections.[8] Due to the tight daily schedule, university students tend to consume high-calorie food selections, fast food snacking, fried food consumption, and low intake of daily fruits and vegetables.[9]especially when transitioning into university life as they become responsible for their daily eating and lifestyles. This study estimates the prevalence of overweight/obesity and explores the eating patterns and lifestyle practices of university students. METHODS: A cross-sectional study was conducted at Universiti Brunei Darussalam (UBD The low intake of vitamin A-rich foods among university students can increase the risk of ocular health disorders, including CVS.[10] Vitamin A is a micronutrient mixture of retinol, retinal, and retinoic acid, along with other components such as β-carotene. The most common animal sources of vitamin A are cod liver oil, liver, butter, cheese, eggs, and fish. In contrast, the most common vegetable sources include sweet potato, carrot, broccoli, sweet red pepper, spinach, and lettuce.[11] Vitamin A is essential for good vision, growth, reproduction, bone metabolism, gene transcription, and immune health.[12] Vitamin A deficiency can exacerbate the symptoms of CVS.[13]pre-school children are the most at-risk population groups for vitamin A deficiency (VAD It has been discovered that carotenoid vitamin therapy increases macular pigment concentrations with concomitant improvements in visual performance; this may be clinically pertinent for treating digital eye strain.[8] This research investigates whether increased screen time and vitamin A-rich food consumption behavior are associated with CVS in preclinical medical students.

## Methods

A cross-sectional, observational, web-based survey was conducted on medical students in the Faculty of Medicine at Universitas Islam Sultan Agung, Semarang, Indonesia. Participants included the third year of preclinical level from a five-year medical education program. This study was conducted in February 2022. Under the assumption of a 95% predictive prevalence, 95% confidence, a 5% margin of error, and a 75% response rate, we determined that a sample size of 95 would be representative of the entire population. The sampling method used a consecutive sampling method. All intended participants were eligible to take part. Exclusion criteria included a previous history of ocular surgery or active ocular diseases. All participants were informed of the purpose of the study, and their participation was voluntary. All respondents provided their informed consent digitally and signed online. The Institutional Review Board (or Ethics Committee) of the Faculty of Medicine at Universitas Islam Sultan Agung (Document No. 45/II/2022/ Komisi Bioetik) approved the research and conducted it following the Declaration of Helsinki.

Using an online survey platform (docs.google.com/forms/), a researcher designed and disseminated the questionnaire to students via WhatsApp groups. The survey consisted of three fundamental sections: demographic data (age and past medical history of ocular disorders), screen time, vitamin A consumption behavior, and computer vision syndrome (CVS) symptoms.

Students were asked to estimate the average amount of time they spent on all digital devices (phone, computer, tablet, television, etc.) per day during the previous week following the beginning of the semester. The screen time for all activities, including work, study, and leisure, was accounted for. The screen time was categorized as 2 h, 2-4 h, 4-6 h, and 6–10 h/day. Vitamin A consumption behavior was asked through a Semi-Quantitative Food Frequency Questionnaire (SQ-FFQ) questionnaire[14]utilisation and validation of food-frequency questionnaires (FFQ with some modifications. The questionnaire consisted of four multiple-choice questions related to the type and frequency of consuming foods that contain vitamin A in the last three months. The vitamin A food sources were selected based on the vitamin A-rich food reported in the literature,[15] using multiple-choice scores of 0,1,2,3 for A, B, C, and D, respectively. The total score of vitamin A consumption attitudes was categorized as low (0-13), moderate (5-8), and good (9-12). Ten self-reported queries were used to validate CVS as a reliable instrument for assessing CVS. The answer is "Yes" and "No". Symptoms are categorized as "Yes" if one of the answers is Yes.

The data from the online survey platform were extracted into Microsoft Excel. The data were analyzed using SPSS version 25.0 (IBM, United States). The Chi-square test examined the relationship between screen time, vitamin A, and

computer vision syndrome symptoms. A *p-value* of 0.05 was considered statistically significant.

## Results

The respondents ranged from 19 to 21, and 84.2% were female. Table 1 depicts respondents' time before a screen, the amount of vitamin A they consumed, and the CVS problems they encountered. In addition to having a daily screen time of over 10 h, 66.3% of respondents reported moderate vitamin A consumption behaviour.

Based on the types of vitamin A-containing foods consumed by the respondents, it can be determined that they consumed fewer vitamin A-containing foods throughout the study. 52.6% of respondents consume vitamin A supplements infrequently, whereas 41.1% consume packaged milk infrequently (Figure 1). As shown in Figure 2, 63.2% of 95 respondents were discovered to have CVS symptoms. Eye fatigue is the most common symptom of CVS among respondents who are preclinical students (62.1%). Double vision and red or watery eyes were reported by 9.5% and 8.4% of respondents, respectively, as the least common CVS symptoms among preclinical students.

The Chi-Square test revealed no association between screen time and CVS ($p>0.05$), although 61.8% of respondents with screen time >10 h/day reported CVS symptoms. Those who consumed less vitamin A, up to 80.8% of respondents, and moderate amounts, up to 58.7% of respondents, reported CVS symptoms. The Chi-Square analysis revealed a correlation ($p<0.05$) between vitamin A consumption and CVS (Table 2). Only one independent variable was associated with the dependent variable, so a multivariate analysis was not conducted.

**Table 1 Screen Time, Vitamin A Consumption Behaviour, and Visual Disturbance Among Respondents**

| Variable | Total respondents (%) |
|---|---|
| Screen time (h/day) | |
| < 2 | 0 (0) |
| 2–4 | 0 (0) |
| 4–6 | 1 (1.1) |
| 6–10 | 19 (18.9) |
| >10 | 76 (80.0) |
| **Vitamin A consumption behaviour** | |
| Less | 26 (27.4) |
| Moderate | 63 (66.3) |
| Good | 6 (6.3) |
| **Computer vision syndrome (CVS) symptoms** | |
| Yes | 60 (63.2) |
| No | 35 (36.8) |

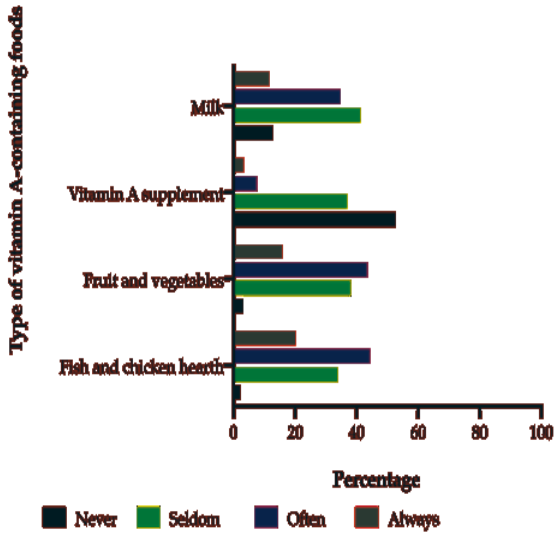


**Figure 1 The Frequency of Consumption of Type of Vitamin A-Containing Foods Consumed by The Respondents**

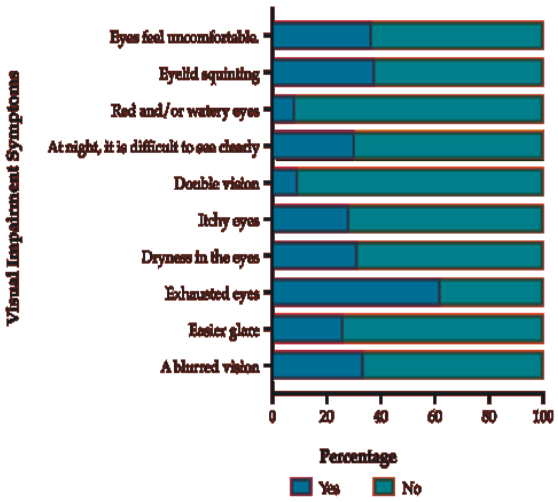


**Figure 2 The Frequency of CVS Symptoms Faced by The Respondents**

**Table 2 Association between Screen Time, Vitamin A Consumption Behavior, and Computer Vision Syndrome (CVS) Symptoms**

| Variable | CVS symptoms (N=95) | | *p* |
|---|---|---|---|
| | Yes | No | |
| **Screen time (h/day)** | | | |
| < 2 | 0 | 0 | 0.304 |
| 2–4 | 0 | 0 | |
| 4–6 | 1 | 1 | |
| 6–10 | 13 | 5 | |
| >10 | 47 | 29 | |
| **Vitamin A consumption behaviour** | | | |
| Less | 21 | 5 | 0.045 |
| Moderate | 37 | 26 | |
| Good | 2 | 4 | |

## Discussion

Computer Visual Syndrome (CVS) refers to visual disturbances resulting from using electronic media, such as computers, laptops, tablets, and smartphones. Eye and head disorders, complaints of pain or headache, dry eyes, irritation, and eye fatigue indicate CVS.[16]so that extensive daily use for both social and professional purposes is now normal. Digital eye strain (DES Excessive screen time, repetitive focusing of the eyes, continuous exposure to blue light, and an unergonomic monitor screen position (too high, too low, too close, too far) for the eyes are the causes of CVS.[17]

This study found no association between screen time and CVS among preclinical Faculty of Medicines students at Universitas Islam Sultan Agung. University students have been documented to develop CVS potentially due to the study burden and heavy use of digital devices, with a reported high prevalence.[18] Preclinical students have a prevalent leading CVS of ocular fatigue, consistent with another study in Nepal and numerous studies.[19] Computer work requires frequent saccadic eye movements (ocular motility), accommodation (continuous focusing), and vergence (alignment demands), which all entail endless relaxation and contraction of the eye muscles.[20] When students spend up to twice as much time in front of a screen, their CVS, such as headaches and itchy eyes, worsen.[6]

In recent years, the function of nutrition in promoting optimal visual performance and the potential consequences of inadequate nutrient intake have become increasingly apparent.[21] Vitamin A deficiency is related to the absence of two significant vitamin A metabolites with very different functions: the chromophore 11-cis-retinal (vitamin A aldehyde) is a crucial component of the visual pigment that mediates phototransduction, whereas the signaling molecule all-trans-retinoic acid regulates the development of various tissues and is essential for immune function. Since humans cannot synthesize vitamin A, they must consume either preformed vitamin A from animal products or carotenoid precursors from plant sources. Acute vitamin A deficiency impairs photoreceptor function and causes night blindness (deficient vision in dim light), whereas chronic vitamin A deficiency causes retinal dystrophy and photoreceptor cell death.[22] CVS in preclinical students during their education is not only caused by the prolonged and extended use of smartphones, tablets, e-readers, and computers. It can be attributed to low levels of vitamin A consumption.[23] This study shows a 6.3% out of 93 who consumed vitamin A-containing foods during their research. This study demonstrates that vitamin A consumption attitudes among university students in the Faculty of Medicine, Universitas Islam Sultan Agung, is significantly ($p<0.05$) associated with CVS, such that the higher the vitamin A intake, the lower the proportion of respondents who experience CVS. This is because of the intensity of the lectures; most students admitted during their study that they consume vitamin-A-rich vegetables and fruits infrequently, preferring to consume fast or instant food).

The rational use of screen time and adopting

ergonomics should be promoted to protect medical students from these CVS.[19] This study is relevant to the previous study reported by Rochmayani and Cahyaningsih (2021) that using the computer for more than 4 h continuously, short (<10 min) break after the computer, the distance of vision less than <50 cm and higher position the top of the monitor than the horizontal height of the eye showed an 83% greater risk of CVS.[24] This study suggests that some measures should be encouraged for students who take online courses at home, such as promoting regular breaks, setting balanced illumination of screen light and room light, and keeping a suitable distance from the screen.[10] To prevent the risk of CVS, policymakers should reduce the length of online classes for students and online work hours for professionals.[6] An adjunctive nutraceutical, especially vitamin A strategies, may impart additional ocular and systemic health benefits to students with CVS. A review on the potential function of micronutrients with nutraceutical properties in the diet reported that antioxidant and immunosuppressive properties of carotenoid-rich fruits and vegetables as a vitamin A source exhibit improved cognitive functioning and overall visual performance, thereby reducing digital eye strain.[24] A placebo-controlled trial that involved 48 healthy young adults with at least 6 h of daily near-field screen time exposure showed that supplementation of macular carotenoids lutein, zeaxanthin, and mesozeaxanthin for 6 months yielded significant improvement in macular pigment optical density (MPOD), overall sleep quality, headache frequency, eye strain, eye fatigue, and all visual performance measures, versus placebo ($p < 0.05$ for all). Increased MPOD significantly improves optical performance and, in turn, improves several undesirable physical outcomes associated with excessive screen time.[25]

An essential limitation of this study is the lack of a control group of university students from non-medical clusters with CVS without longer screen time and vitamin A deficiency. Observing the CVS symptoms among medical students with or without vitamin A deficiency could be more helpful in clarifying the pathogenesis of CVS. In addition, students' daily digital screen time consisted of both studying and non-studying activities, so the effect of education on the prevalence of CVS cannot be determined with precision. The function of vitamin A supplementation in the amelioration of CVS can be investigated in future research.

It can be concluded that screen time and vitamin A-rich food consumption behavior are associated with CVS among preclinical students. This study highlights the need for strategies to manage screen time and vitamin A consumption among students at risk of developing CVS symptoms.

## Acknowledgments

The authors are grateful to anonymous referees for their useful sug-gestions and reviewing the manuscript. This study was supported by the research grant from Universitas Islam Sultan Agung with contract number 246/B.1/SA-LPPM/VII/2021.

## References

1. Coles-Brennan C, Sulley A, Young G. Management of digital eye strain. Clin Exp Optom. 2019;102(1):18–29. doi:10.1111/cxo.12798
2. Lema AK, Anbesu EW. Computer vision syndrome and its determinants: A systematic review and meta-analysis. SAGE Open Med. 2022;10:205031212211424. doi:10.1177/20503121221142402
3. Munshi S, Varghese A, Dhar-Munshi S. Computer vision syndrome—A common cause of unexplained visual symptoms in the modern era. Int J Clin Pract. 2017;71(7):e12962. doi:https://doi.org/10.1111/ijcp.12962
4. Choi JH, Li Y, Kim SH, et al. The influences of smartphone use on the status of the tear film and ocular surface. PLoS One. 2018;13(10):e0206541. https://doi.org/10.1371/journal.pone.0206541
5. Toh SH, Coenen P, Howie EK, Mukherjee S, Mackey DA, Straker LM. Mobile touch screen device use and associations with musculoskeletal symptoms and visual health in a nationally representative sample of Singaporean adolescents. Ergonomics. 2019;62(6):778–93. doi:10.1080/00140139.2018.1562107
6. Ganne P, Najeeb S, Chaitanya G, Sharma A, Krishnappa NC. Digital eye strain epidemic amid COVID-19 pandemic – a cross-sectional survey. Ophthalmic Epidemiol. 2021;28(4):285–92. doi:10.1080/09286586.2020.1862243
7. Wang L, Wei X, Deng Y. Computer vision syndrome during SARS-CoV-2 outbreak in

University Students: a comparison between online courses and classroom lectures. Front public Heal. 2021;9:696036. doi:10.3389/fpubh.2021.696036
8. Lem DW, Gierhart DL, Davey PG. Can nutrition play a role in ameliorating digital eye strain?. Nutrients. 2022;14(19): 4005. doi:10.3390/nu14194005
9. Chen Yun T, Ahmad SR, Soo Quee DK. Dietary habits and lifestyle practices among University Students in Universiti Brunei Darussalam. Malaysian J Med Sci. 2018;25(3):56–66. doi:10.21315/mjms2018.25.3.6
10. Atmadja TFA, Yunianto AE, Yuliantini E, Haya M, Faridi A, Suryana S. Gambaran sikap dan gaya hidup sehat masyarakat Indonesia selama pandemi Covid-19. AcTion Aceh Nutr J. 2020;5(2):195–202. doi:10.30867/action.v5i2.355
11. Prasetya H, Isradji I, Suparmi S, et al. Perbandingan aktivitas antioksidan dan toksisitas antara drop vitamin a dari karotenoid kulit pisang ambon dan ß-Karoten. 2017;49(1):1–7. http://dx.doi.org/10.15395/mkb.v49n1.981
12. Alanazi SA, El-Hiti GA, Al-Baloud AA, et al. Effects of short-term oral vitamin A supplementation on the ocular tear film in patients with dry eye. Clin Ophthalmol. 2019;13:599–604. doi:10.2147/OPTH.S198349
13. Yisak H, Elmneh R, Taklual W, Ewunetei A, Kefale B. Prevalence and associated factors of clinical vitamin A deficiency among pre-school children 1-5 years of age in rural kebeles in farta district, south gondar zone, Ethiopia: A mixed methods study. J Multidiscip Healthc. 2020;13:1191–201. doi:10.2147/JMDH.S279571
14. Cade JE, Burley VJ, Warm DL, Thompson RL, Margetts BM. Food-frequency questionnaires: a review of their design, validation and utilisation. Nutr Res Rev. 2004;17(1):5–22. doi:DOI: 10.1079/NRR200370
15. Siagian SD, Herlina S, Sidoretno WM. Kandungan Vitamin a Pada Buah Pepaya Hijau: Solusi Meningkatkan Produksi ASI. Pros Semin Nas Poltekkes Karya Husada Yogyakarta Tahun 2019. 2019;1(1):129–34.
16. Sheppard AL, Wolffsohn JS. Digital eye strain: prevalence, measurement and amelioration. BMJ Open Ophthalmol. 2018;3(1):e000146. doi:10.1136/bmjophth-2018-000146
17. Li L, Zhong H, Li J, Li C-R, Pan C-W. Incidence of myopia and biometric characteristics of premyopic eyes among Chinese children and adolescents. BMC Ophthalmol. 2018;18(1):178. doi:10.1186/s12886-018-0836-9
18. Salote A, Jusuf H, Amalia L. Hubungan lama paparan dan jarak monitor dengan gangguan kelelahan mata pada pengguna komputer. J Heal Sci Gorontalo J Heal Sci Community. 2020;4(2):104–21. doi:10.35971/gojhes.v4i2.7842
19. Solia E, Nikolaou A, Kotsira T, Filippou D. Medical Students and Computer Vision Syndrome. A review. Top Biomed Res Educ. Published online April 1, 2023.
20. Alemayehu AM, Alemayehu MM. Pathophysiologic mechanisms of computer vision syndrome and its prevention: review. World J Ophthalmol Vis Res. 2019;2(5):1–7. doi:10.33552/wjovr.2019.02.000547
21. De Sanctis V, Soliman A, Alaaraj N, Ahmed S, Alyafei F, Hamed N. Early and long-term consequences of nutritional stunting: from childhood to adulthood: early and long-term consequences of nutritional stunting. Acta Biomed Atenei Parm. 2021;92(1 SE-UPDATE ON ADOLESCENT MEDICINE):11346. doi:10.23750/abm.v92i1.11346
22. Dewett D, Lam-Kamath K, Poupault C, Khurana H, Rister J. Mechanisms of vitamin A metabolism and deficiency in the mammalian and fly visual system. Dev Biol. 2021;476:68–78. doi:10.1016/j.ydbio.2021.03.013
23. Cantó-Sancho N, Sánchez-Brau M, Ivorra-Soler B, Seguí-Crespo M. Computer vision syndrome prevalence according to individual and video display terminal exposure characteristics in Spanish university students. Int J Clin Pract. 2021;75(3):e13681. doi:https://doi.org/10.1111/ijcp.13681
24. Rochmayani DS, Cahyaningsih O. Risk factors for the incidence of computer vision syndrome (CVS) in lecturers during the online learning period. J Heal Educ. 2021;6(2):65–72. doi:10.15294/jhe.v6i2.47513
25. Stringham JM, Stringham NT, O'Brien KJ. Macular carotenoid supplementation improves visual performance, sleep quality, and adverse physical symptoms in those with high screen time exposure. Foods. 2017;6(7):47. doi:10.3390/foods6070047

RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v56.3249
Majalah Kedokteran Bandung. 2024;56(1):35–41

Majalah Kedokteran Bandung (MKB)

Received: February 16, 2023
Accepted: May 10, 2023
Available online: March 31, 2024

# Dysobinol Extracted from *Chisocheton Macrophyllus* Triggers Proliferation Inhibition, Potential Apoptosis, and Cell Cycle Arrest of He La Cancer Cell Lines

**Shabarni Gaffar,[1,2] Ersanda Hafiz,[1] Hesti Lina Wiraswati,[3] Safri Ishmayana,[1] Nurlelasari Nurlelasari[1,4]**
[1]Departement of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Indonesia
[2]Graduate School, Universitas Padjadjaran, Indonesia
[3]Department of Biomedical Sciences, Faculty of Medicine Universitas Padjadjaran
[4]Research Center of Natural Product, Universitas Padjadjaran, Indonesia

**Abstract**

Dysobinol is a new limonoid from *C. macrophyllus* seeds reported to have an anticancer activity. This study aimed to determine the cytotoxic activity of Dysobinol against HeLa cancer cell lines and evaluate its mechanism of action by determining the expression level of several carcinogenesis genes related to apoptosis and cell cycle. In this experimental study, the cytotoxic activity was determined using the MTS assay and gene expression by real-time reverse transcriptase PCR. The result shows that Dysobinol has an anticancer activity in a dose and time-dependent manner against HeLa cells and was categorized as toxic with $IC_{50}$ values of 52.92, 52.70, and 14.96 µg/ml for 24, 48, and 72 hours, respectively. Dysobinol significantly increased the expression of Bax, Cas-8, and Cas-3 and decreased the expression of Cyc D1 at both doses ($IC_{50}$ and 2x $IC_{50}$) but only high doses (2x $IC_{50}$) could affect Cas9 and NF-κB expressions, indicating that Dysobinol can induce apoptosis via the extrinsic pathway and inhibits the cell cycle through the Cyc D1 regulator. Dysobinol has the potential to be developed as a chemotherapy drug or an adjuvant agent for cervical cancer treatment.

**Keywords:** Apoptosis, cell cycle arrest, cytotoxic activity, dysobinol, HeLa cell line

## Introduction

Meliaceae family plants in tropical and subtropical areas contain secondary metabolites, namely terpenoids, limonoids, alkaloids, flavonoids, and phenols, which have biological activities, such as anticancer, antibacterial, antifungal, antimalarial, and antiviral.[1,2] The Chisocheton genus is the second largest genus of the Meliaceae family which is known to contain limonoid, tirukalan, resin, lupan, oleanan, steroid, sesquiterpene, anthraquinone, alkaloid, coumarin, and simple phenolic compounds. It has been used traditionally to treat several diseases, such as stomach pain, back pain, fever, rheumatism, and malaria.[3] Recently, secondary metabolites have been extracted from the Chisocheton genus with biological activity, including anticancer, dysobinol, disobinin, nimonol, and 7α-hydroxyneotrisilenone.[4]

Limonoids are triterpenoid derivative compounds that are oxygenated and lose four-terminal carbon atoms in the side chain to form a furan ring.[5] These compounds display cytotoxic activity,[6,7] antiplasmodial,[8] antimycobacterial,[7] antiviral,[9] insecticidal,[10] antifungal,[11] anti-inflammatory properties,[12] induce apoptosis,[13] and inhibit lipid droplet accumulation.[14] Dysobinol (Figure 1) is a new limonoid compound that belongs to the group with intact A, B, C, and D rings and α and β unsaturated ketones in ring A.[4] The presence of α, β unsaturated ketones in ring A has an essential role in its anticancer activity.[3]

The search for anticancer drugs today is mainly directed at finding natural compounds that can specifically inhibit the cell cycle or induce apoptosis in cancer cells. Several proteins in the signaling pathway are overexpressed in cancer cells, such as the Her2, Ras, and MAPK proteins, while apoptotic proteins are suppressed so cancer cells grow uncontrolled.[15]

Finding natural compounds for cancer cell treatment is important because its have a lower risk of toxicity and side effects compared to

**Corresponding Author:**
Shabarni Gaffar,
Departement of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jalan Ir. Soekarno Km 21, Jatinangor, Sumedang, West Java, Indonesia
Email: shabarni.gaffar@unpad.ac.id

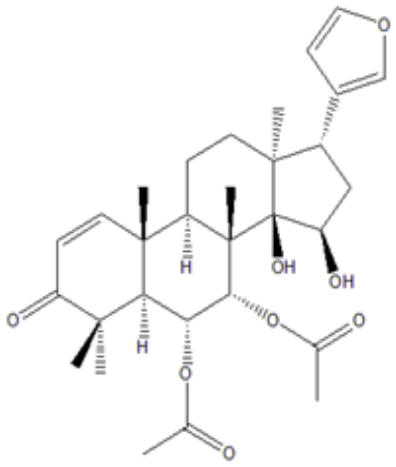


**Figure 1 Structure of Dysobinol from seed of C. *macrophyllus*[4]**

synthetic compounds. Additionally, natural compounds are often more accessible and affordable than synthetic drugs, especially in developing countries where access to modern cancer treatments may be limited. Current cancer treatments such as chemotherapy, radiation therapy, and targeted therapy often have significant side effects and may not be effective for all types of cancer. Chemotherapy, targets both cancer cells and healthy cells, which can lead to various side effects such as nausea, vomiting, hair loss, and fatigue. Radiation therapy can cause damage to healthy tissue and organs, leading to long-term complications. Targeted therapy drugs can be effective but are often very expensive and may not be accessible to all patients. Therefore, finding natural compounds that can specifically target cancer cells while leaving healthy cells unharmed would be a major breakthrough in cancer treatment.[13]

The incidence of cervical cancer in Indonesia is high, and 50% of those affected die.[16] HeLa cells are cervical cancer cells infected with HPV-18 and contain a wild-type p53 protein, but p53 expression is low. The tumor suppressor p53 is a transcription factor that regulates about 500 gene targets in controlling various cellular processes such as cell cycle arrest, cell aging, DNA repair, metabolic adaptation, and apoptosis,[17,18] This study determined the cytotoxic activity of Dysobinol against HeLa cells, and the expression of genes related to apoptosis and the cell cycle that play a role in carcinogenesis, such as p53, Bcl-2, Bax, Cas-9, Cas-8, Cas-3, Cyc D1, and NF-κB.

Apoptosis is also influenced by changes in the shape of the mitochondrion and the availability of the Bcl-2 protein family. Bax and Bcl-2 belong to the Bcl-2 protein family that regulates the permeability of the mitochondrial membrane, resulting in the release of cytochrome-C. The release of cytochrome-C will activate caspase-9, initiates of the intrinsic apoptosis pathway.[19]

Apoptosis is also closely related to the cell cycle that is linked to cell cycle regulators protein, one of which is cyclin D1. Cyclin D1 is a proto-oncogene that is often overexpressed in cancer patients. Excessive expression of cyclin D1 results in hyperphosphorylation of the famous transcriptional regulator, retinoblastoma protein (RB or pRB), which play an important role in the cell cycle.[20] One mechanism that causes high levels of cyclin D1 is the signalling of the transcription factor NF-κB. NF-κB activates the *cyclin D1* gene and thus plays a role in regulating the cell cycle. NF-κB is a transcription factor that controls inflammation, immunity, cell proliferation, differentiation, and cell survival.[21]

The objective of this study is to determine the cytotoxic activity of Dysobinol against HeLa cancer cell lines and evaluate its mechanism of action by determining the expression level of some carcinogenesis genes related to apoptosis and the cell cycle. This study will provide information of the potency of Dysobinol for chemotherapy or as an adjuvant agent for the treatment of cervical cancer, especially for patients who do not respond to current treatments or who are unable to access them.

## Methods

The Dysobinol used in this study was a pure compound, and its structure was determined spectroscopically by Nurlelasari et al. (2017). HeLa cell lines were from the culture collection of Central Laboratory Universitas Padjadajaran. RPMI-1640, fetal bovine serum (FBS), 0.25% Trypsin-EDTA solution, DMSO, chloroform, isopropyl alcohol, 75% ethanol, DEPC-treated water, and all antibiotics were purchased from Sigma Aldrich. The MTS assay kit (CellTiter 96® $AQ_{ueous}$ One Solution Cell Proliferation Assay was from Promega, TRIsure™ was from Meridian-Bioscience, and SensiFAST™ SYBR® No-ROX One-Step Kit from Bioline.

Cell viability was assessed using the colorimetric MTS assay. HeLa cells were grown until 80% confluent in complete medium (RPMI supplemented with 10% PBS, penicillin, and streptomycin), harvested and counted, then

**Table 1 Primers Sequences Used in This Study**

| Genes | Forward Primer | Reverse Primer |
|---|---|---|
| p53 | 5'-CCT CAG CAT CTT ATC CGA G -3' | 5'-TGG ATG GTG GTA CAG TCA G -3' |
| Bax | 5'-GCG AGT GTC TCA AGC GCA TC -3' | 5'-CCA GTT GAA GTT GCC GTC AG - 3' |
| Bcl-2 | 5'-CAG GCC GGC GAC GAC TTC TC -3' | 5'-TCC CGG TTG ACG CTC TCC AC -3' |
| CycD1 | 5-'AGG CGG AGG AGA ACA AAC AC-3' | 5'-GTG AGG CGG TAG TAG GAC AG -3' |
| Cas-3 | 5'- GTC GAT GCA GCA AAC CTC AG -3' | 5'-TTC TAC AAC GAT CCC CTC TG - 3' |
| Cas-8 | 5'- CAT CCA GTC ACT TTG CCA GA -3' | 5'- GCA TCT GTT TCC CCA TGT TT -3' |
| Cas-9 | 5'-CCT CAC CCT GCC TTA TCT TG -3' | 5'-TCC ACG GCA TTC ATC TGT CC -3' |
| $NF_KB$ | 5'-CAA GGC AGC AAA TAG ACG AG -3' | 5'-GGG CAT TTT GTT GAG AGT TAG -3' |
| B-act | 5'-GAT CAT TGC TCC TCC TGA GC -3' | 5'-TAG AAG CAT TTG CGG TGG AC -3' |

diluted with complete culture medium and transferred into 96-well plates with 1.7 x 104 cells/well and incubated for 24 hours. The media was replaced, and cells were incubated for 24 hours with increasing concentrations of Dysobinol (1.95; 3.91; 7.8; 15.63; 31.25; 62.5; and 125 µg/mL) in DMSO (final concentration <0.2%). Doxorubicin (0.75–48 µg/mL) was used as a positive control, medium and DMSO 0.1% as negative control. All experiment was done in three replicated. All samples were incubated for 24, 48, and 72 hours at 37°C in a 5% CO2 incubator (NuAire, Plymouth, MN, USA). After incubation, 20 µL MTS reagent was added into each well and incubated for 4 hours at 37°C. Then, 10% SDS was added to stop the MTS reduction reaction and the absorbance was measured at 490 nm using a 96-well plate reader (NanoQuant, TECAN). The % of viable cells was calculated based on equation: $(A_{sample}-A_{medium}/A_{DMSO}-A_{medium})$ x100%. By plotting the % of viable cells (y) vs concentration (x), the $IC_{50}$ value will be obtained at the 50% of viable cell.

HeLa cells were grown in 12-well plates (1.7 x $10^4$ cells/well) and incubated for 24 hours or until 80% confluent. The concentration of Dysobinol used in this study was the $IC_{50}$ and 2x $IC_{50}$ values of the 24-hour incubation. The total RNA was extracted using a TRI-sure™ reagent (Meridian-Bioline) according to the manufacturer's protocol and the concentration was measured using a Multimode reader (Nano Quant, Tecan).

Quantitative PCR was performed using the SensiFAST™ SYBR® No-ROX One-Step Kit (Bioline) and 5 ng/µL of RNA template per reaction according to the manufacturer's instructions on an AriaMx *Real-Time* qRT-PCR (Agilent). The cycling conditions used were two-step cycling, the first cycle was 1 cycle at 45ºC for 10 minutes (reverse transcription) and the second step was 1 cycle at 95ºC for 2 minutes, and 40 cycles at 95ºC for 5 seconds, 60ºC for 20 seconds. The primers were designed based on the gene sequences available in NCBI (Table 1). Genes expression was quantified using the $2^{-\Delta\Delta CT}$ method and the housekeeping gene β-actin.[22]

The data presented are means of three independent experiments. The qPCR results were analyzed by Minitab version 17 using ANOVA analysis with a 95% confidence level. The difference was considered statistically significant at the p-value <0.05. Tukey Post hoc test was used to compare the means of every treatment to the standards of every other treatment.

## Results

The percentage of viable cells with the administration of Dysobinol decreased with increasing Dysobinol concentrations and the $IC_{50}$ value decreased with the longer incubation time. The $IC_{50}$ of Dysobinol against HeLa cells were: 52.92; 52.70 and 14.96 µg/ml with incubation times of 24, 48, and 72 hours, respectively (Figure 2). Observation of the cells under a light microscope shows that Dysobinol altered cell morphology. It can be seen that high Dysobinol caused more cells undergoes morphological alteration, indicated by small brown rounded form cells looked cloudy and floated compared to control cells which were elongated and attached to a petri dish. Cell turbidity also decreased with increasing dysobinol concentration (data was not shown). There was a decrease in the percentages of living HeLa cells along with the increasing concentration of Dysobinol and the length of the incubation time.

Based on the National Cancer Institute, a

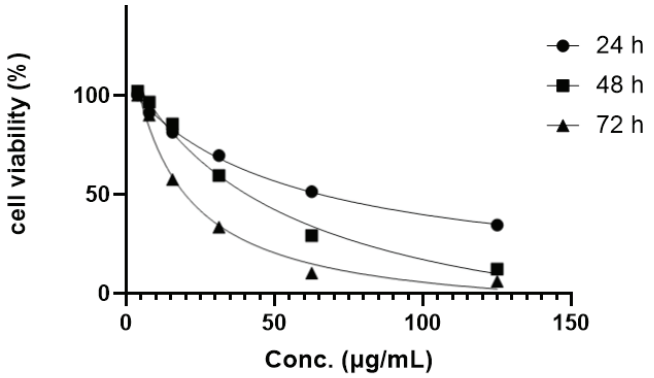


**Figure 2 HeLa Cell Viability Curve That Treated with Dysobinol at Different Incubation Time. The cell viability of HeLa cells treated with Doxorubicin. Data represent the mean ± SD of the three independent experiments**

powerful cytotoxic effect was characterized by $IC_{50}$ <21 µg/ml, moderate cytotoxic effect by $IC_{50}$ of 21-200 µg/ml, and weak cytotoxic effect by $IC_{50}$ of 201-500 µg/ml; meanwhile, IC>500 µg/ml are considered non-toxic. Based on its $IC_{50}$ value, the cytotoxicity of Dysobinol was considered moderate. Meanwhile, $IC_{50}$ of doxorubicin, a well-known chemotherapy agent to the HeLa cells is 2.13 µg/mL.

The qPCR results are depicted in Figure 3. Based on one-way ANOVA followed by the Tukey Post hoc test, there was a significant change in the expression of Bax (3.12x and 3.28x), Cas-8 (3.30x and 2.95x), Cas-3 (4.31 and 3.09x), and Cyc D1 (-0.46x and -0.41x) in Dysobinol-treated HeLa cells. Furthermore, the change in Cas-9 and $NF_KB$ gene expression only reached significance at the higher dose of 2x $IC_{50}$ of Dysobinol. However, there were no significant changes in the expression of the transcription factor p53 and the anti-apoptosis gene Bcl-2. In the qPCR, doxorubicin is not used as a positive control, because its mechanism is well-known and cannot be compared to Dysobinol.

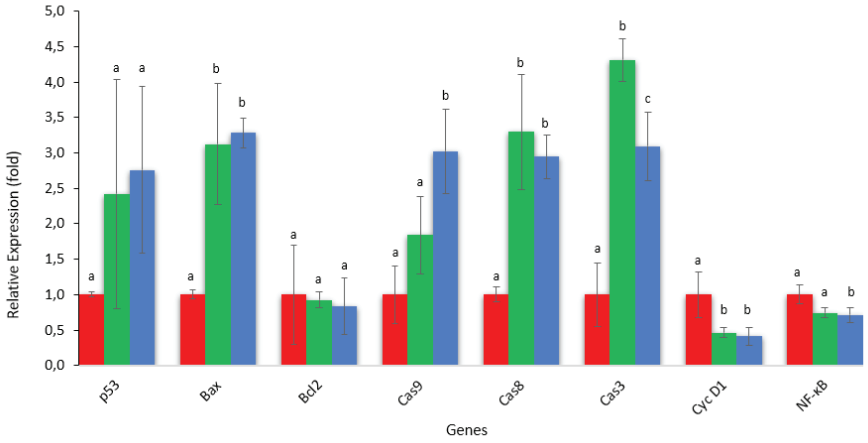


**Figure 3 Changes in the Expression of p53, Bax, Bcl-2, Cas-9, Cas-8, Cas-3, Cyc D1, and $NF_KB$ Genes After Administration of Dysobinol to HeLa cells for 24 Hours**

Red = Control Cells (without treatment), Green = Dysobinol $IC_{50}$, Blue = Dysobinol 2x $IC_{50}$. Gene expression was normalized to ß-actin. Data are presented as the mean ± SD and representative of three experiments. Bars that do not share a letter are significantly different for each gene based on one-way ANOVA, followed by the Tukey post hoc test with a significance level of = 0.05.

## Discussion

Dysobinol has a similar structure to disobinin derived from *C. siamensis* which is toxic to NCI-H187, KB, and MCF-7 cancer cell lines with an $IC_{50}$ of 1.67, 3.17, and 2.15 µg/ml, respectively.[9] However, the $IC_{50}$ of Dysobinol against the HeLa cell line was not as high as Disobinin. Disobinin has two ester groups (acetyl group) on the B-ring, a double bond on the D-ring, and a methylene group (CH2) at the C-16,[23] whereas Dysobinol has no double bond on the D-ring and two hydroxyl groups on the D-ring at the C-14 and C-15 positions. The existence of these two hydroxyls may reduce its anticancer activity and the presence of double bonds in disobinin compounds plays an important role in anticancer activity.[23]

There was a significant increase in the expression of Caspase-8 (extrinsic apoptosis markers) and the executor protein Cas-3 (both extrinsic and intrinsic apoptosis markers). Although the expression at 2x $IC_{50}$ concentration decreased in both genes, this did not reach significance but suggests that Dysobinol has the potential to trigger apoptosis via the extrinsic pathway. Cas-8 is activated due to the presence of death signals, TNF, and FAS, which are recognized by death receptors. This binding causes a change in shape and oligomerization of the receptors. The adapter protein recognizes changes in active receptors so that it is directed to procaspase 8 aggregation for Cas-8 activation. In some cells, Cas-8 can directly cut Cas-3 as the executor caspase.[24]

Dysobinol also significantly decreases the expression of Cyc D1, a cell cycle regulator gene in HeLa cells, and thus has the potential to inhibit the cell cycle by reducing Cyc D1 expression. However, this result should be confirmed by western blot analysis. Higher Cyc D1 expression is found in several human cancers such as breast cancer and cervical cancer.[25] Overexpression of cyclin D1 results in dysregulated CDK activity, rapid cell growth under conditions of restricted mitogenic signaling, bypassing key cellular checkpoints, and ultimately, neoplastic growth.[25] One of the cancer therapies targets Cyc D1 by inhibiting the formation of the Cyclin-CDK complex which phosphorylates the target protein such as retinoblastoma (RB) protein that plays an important role in the cell cycle. The decrease in Cyc D1 expression will trigger a checkpoint in the G1 phase due to the unavailability of several proteins required for DNA replication and the cell cycle. Therefore, inhibition of the cell cycle in the G1 phase will allow DNA repair, thereby maintaining the integrity of chromosomes and improving the survival of damaged cells.[19]

Dysobinol had no significant effect on increasing p53 expression and was unable to normalize the function of p53 which is the target of HPV virus E6 protein degradation. However, several studies have shown that apoptosis induction and cell cycle inhibition can still occur without p53, one of which is with sufficient Bax protein concentration through extrinsic pathways, and lowering Cyclin expression. Cyclin B1 and CDK1 are independent of p53.[27]

Dysobinol only had a significant effect on Bax expression. Bax and Bcl-2 are members of the Bcl-2 protein family that plays an important role in the regulation of the intrinsic pathway of apoptosis along with the release of procaspase-9 from the mitochondria. The translocation of Bax and the cleavage of Caspase 3 by Caspase-9 initiates the intrinsic pathway. Bax and Bak act as executor proteins for mitochondrial outer membrane permeability (MOMP),[19] therefore, the effect of increased Bax expression should be further investigated by flow cytometry. However, the small changes in Cas-9 expression and the insignificant changes in Bcl-2 expression indicate that Dysobinol does not trigger intrinsic pathway apoptosis.

NF-κB is also a family of transcription factors that have essential regulatory functions in inflammation, the immune response, cell proliferation, and apoptosis. The decrease in NF-κB expression was only significant at 2x IC50, indicating that Dysobinol has no significant effect on the expression of proteins that regulate the cell cycle and apoptosis through the NF-κB pathway, which is in line with the absence of a significant decrease in Bcl-2 expression.

In conclusion, Dysobinol has a dose- and time-dependent cytotoxic effect on HeLa cells and the potential to trigger apoptosis via the extrinsic pathway and inhibit the cell cycle, therefore it could be investigated as a potential drug for chemotherapy or as an adjuvant agent for the treatment of cervical cancer.

## Acknowledgments

This research was funded by Universitas Padjadjaran, grant number 1427/UN6.3.1/LT/2020 to S.G. This research was supported by Universitas Padjadjaran. We thank to Ghina Uli Felicia for assistant with methodology.

## References


1. Perera WPRT, Liyanage JA, Dissanayake KGC, Gunathilaka H, Weerakoon WMTDN, Wanigasekara DN, et al. Antiviral potential of selected medicinal herbs and their isolated natural products. Biomed Res Int. 2021;7872406:1–18.
2. Happi GM, Nangmo PK, Dzouemo LC, Kache SF, Kouam ADK, Wansi JD. Contribution of Meliaceous plants in furnishing lead compounds for antiplasmodial and insecticidal drug development. J Ethnopharmacol. 2022;285:114906.
3. Shilpi JA, Saha S, Chong SL, Nahar L, Sarker SD, Awang K. Advances in chemistry and bioactivity of the genus Chisocheton Blume. Chem Biodiv. 2016;13(5):483–503.
4. Nurlelasari N, Katja DG, Harneti D, Wardayo MM, Supratman U, Awang K. Limonoids from the seeds of Chisocheton macrophyllus. Chem Nat Comp. 2017;53(1):83–7.
5. Zhang Y, Xu H. Recent progress in the chemistry and biology of limonoids. Royal Soc. of Chem. Adv. 2017;7(56):35191–220.
6. Chen J, Fan X, Zhu J, Song L, Li Z, Lin F, et al. Limonoids from seeds of Azadirachta indica A. Juss. and their cytotoxic activity. Acta Pharmaceutica Sinica B**.** 2018;8(4):639–44.
7. Maneerat W, Laphookhieo S, Koysomboon S, Chantrapromma K. Antimalarial, antimycobacterial, and cytotoxic limonoids from Chisocheton siamensis*.* Phytomed 2008;15(12):1130–4.
8. Mohamad K, Hirasawa Y, Litaudon M, Awang K, Hadi AHA, Takeya K, Ekasari W, Widyawaruyanti A, Zaini NC, Morita H. Ceramicines B–D, New antiplasmodial limonoids from Chisocheton ceramicus. Bioorg Med Chem. 2009;17(2):727–30.
9. Inada A, Somekawa M, Murata H, Nakanishi T, Takuda H, Nishino S, et al. Phytochemical studies on Meliaceous plants. VIII. Structures and inhibitory effects on Epstein-barr virus activation of triterpenoids from leaves of Chisocheton macrophyllus King. Chem Pharm Bull. 1993;41(3):617–19.
10. Chong SL, Hematpoor A, Hazni H, Sofian-Azirun M, Lioudon M, Supratman U, Murata M, Awang K. Mosquito Larvacidal limonoids from the fruits of Chisocheton Erythrocarpus Hiern. Phytochem Lett. 2019;30:69–73.
11. Bordoloi M, Saikia B, Mathur RK, Goswami BN. A Meliacin from Chisocheton panniculatus. Phytochem. 1993;34(2):583–84.
12. Yang MH, Wang JG, Luo JG, Wang XB, Kong LY. Chisopanins A-K, 11 new Protolimonoids from Chisocheton paniculatus and their anti-inflammatory activities. Bioorg Med Chem, 2011;19(4):1409–17.
13. Tasyriq M, Najmuldeen IA, In LLA, Mohamad K, Awang K, Hasima N. 7α-hydroxy-β-sitosterol from Chisocheton tomentosus induces apoptosis via dysregulation of cellular Bax/Bcl-2 ratio and cell cycle arrest by downregulating ERK1/2 activation. Evid Based Complement Alternat Med. 2012;12(2):1–12.
14. Wong CP, Kaneda T, Hadi AHA, Morita H. Ceramicine B, A limonoid with anti-lipid droplets accumulation activity from Chisocheton ceramicus. J Nat Med**.** 2014;68(1):22–30.
15. Braicu C, Buse M, Busuioc C, Drula R, Gulei D, Raduly L, Rusu A, Irimie A, Atanasov AG, Slaby O, Ionescu C, Berindan-Neagoe I. A comprehensive review on MAPK: A promising therapeutic target in cancer. Cancers (Basel). 2019;11(10):1618:1–25.
16. Gondhowiardjo S, Christina N, Ganapati NPD, Hawariy S, Radityamurti F, Jayalie VF, et al. Five-year cancer epidemiology at the National Referral Hospital: Hospital-based cancer registry data in Indonesia. JCO Glob Oncol. 2021;7:190–203.
17. Aubrey BJ, Kelly GL, Janic A, Herald MJ, Strasser A. How does p53 induce apoptosis, and how does this relate to p53-mediated tumor suppression? Cell Death Differ. 2018;25:104–113.
18. Chimento A, De Luca A, D'Amico M, De Amicis F, Pezzi V. The involvement of natural polyphenols in molecular mechanisms inducing apoptosis in tumor cells: A promising adjuvant in cancer therapy. Int J Mol Sci. 2023;24(2):1680.
19. Pecorino L. Molecular Biology of Cancer: Mechanisms, Targets, and Therapeutics, Oxford University Press, 5rd ed. 2021. p. 149–60.
20. Phadwal K, Tang QY, Luijten I, Zhao JF, Corcoran B, Semple RK, Ganley IG, MacRae VE. p53 regulates mitochondrial dynamics in vascular smooth muscle cell calcification. Int J Mol Sci. 2023;13;24(2):1643.
21. Poladian N, Orujyan D, Narinyan W, Oganyan AK, Navasardyan I, Velpuri P, Chorbajian A, Venketaraman V. Role of NF-κB during *Mycobacterium tuberculosis* Infection. Int J Mol Sci*.* 2023;24(2):1772.
22. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real time

quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods. 2001;25:402–8.
23. Venkata KCN, Rathinavelu A, Bishayee A. Chapter 11-Limonoids: structure-activity relationship studies and anticancer properties. Studies Nat Prod Chem. 2019;59:375–99.
24. Chakravorty A, Sugden B. Long-distance communication: Looping of human papillomavirus genomes regulates expression of viral oncogenes. PLoS Biol. 2018; 16(11):e3000062.1–7.
25. Wen N, Bian L, Gong J, Meng Y. Overexpression of cell-cycle related and expression-elevated protein in tumor (CREPT) in malignant cervical cancer. J Int Med Res**.** 2020;48(1):1–7.
26. Jun SY, Kim J, Yoon N, Maeng LS, Byun JH. Prognostic potential of cyclin D1 expression in colorectal cancer. J Clin Med. 2023:10;12(2):572.
27. Mandapati A, Lukong KE. Triple negative breast cancer: approved treatment options and their mechanisms of action. J Cancer Res Clin Oncol. 2022;1–19.

RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v56.2657
Majalah Kedokteran Bandung. 2024;56(1):42–47

Majalah Kedokteran Bandung (MKB)

Received: January 3, 2022
Accepted: February 28, 2023
Available online: March 31, 2024

# Impact of Covid-19 Pandemics on Urology Practices and Residency Training in an Indonesian Tertiary Hospital

**Christopher Kusumajaya, Tjahjodjati**
Department of Urology, Faculty of Medicine Universitas Padjadjaran
Dr. Hasan Sadikin General Hospital, Bandung, Indonesia

**Abstract**

The World Health Organization (WHO) declared Covid-19 as a global pandemic on March 11, 2020. Alterations in health service provisions must be applied to maintain prime services and decrease the number of healthcare workers exposure to Covid-19 by reducing the number of patients and workload, as well as cancelling elective surgeries. This study aimed to describe the urological services and residency training program during a Covid-19 year at Dr. Hasan Sadikin General Hospital, Bandung, which is a tertiary health center and teaching hospital in Indonesia. This was a comparative retrospective study conducted from January 2019 to December 2020 that compared the number of patients in inpatient and outpatient settings and emergency department, as well as surgical procedures performed before and during Covid-19 pandemic. Data were obtained from the administration department of the hospital, showing a reduction of 40,94%, 7.39%, 32.3%, and 53.89% of total inpatients, outpatients, emergency cases, and surgical procedures, respectively, in the urology department of the hospital when compared to the previous year. The number of surgeries that could be performed by residents was reduced by 30-60%. This current study showed the decreased number of patients and urological operative procedures during COVID-19 pandemic. This is assumed to give negative impacts to the urological residency training due to the limited exposure to variety of cases and surgical skill procedures.

**Keywords:** Covid 19 pandemic, residency training, urology practices

## Introduction

In Indonesia, the first Covid-19 case was confirmed on March 2nd 2020, and by March 11th 2020 Word Health Organization (WHO) declared Covid-19 a global pandemic.[1,2] Covid-19 which was first discovered in December 2019 in Wuhan, China, is a disease caused by a single-stranded RNA virus, also known as coronavirus 2 (SARS-CoV-2). SARS-CoV-2 lead to various clinical manifestations from mild disease of upper respiratory tracts to severely acute respiratory distress syndrome (ARDS). Transmissions of COVID-19 can occur via droplets and direct contact of human-to-human. Several individuals with Covid-19 infection can be asymptomatic and act as carrier of the disease. Since it was first discovered in Wuhan, Covid-19 global cases have continued to increase.[2,3] This unprecedented public health emergency has changed many aspects around the world including socioeconomic, education, and especially the healthcare systems. According to the WHO, protecting the health and lives of health care providers as the frontliners is critical to enabling a better global response.

Alterations of health services should be done to maintain prime services and reduce the number of healthcare workers exposure against Covid-19 by reducing the number of patients, workloads, and cancellation of elective operations.[2] These decisions resulted in changes in all departments such as the suspension of any outpatient activities, limiting the amount of procedures, and reorganizing activities in the wards, and with no exception, the urology department.[4] Surgery is the basis of a health system with elective and emergency procedures that contribute to the health of the population. Meanwhile the management of Covid-19 does not only focused on the field of urological surgery, furthermore the operating room is an area that is

**Corresponding Author:**
Christopher Kusumajaya,
Department of Urology, Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia,
Email: ck.uro@yahoo.com

susceptible to transmission of respiratory tract infections.[3] A study showed a reduction in the number of urological surgeries from 79.3% to 42% post pandemic. Approximately 33.3% of patients with age of 60 years and over postponed their scheduled surgeries.[5,6] Based on Rasyid et al., elective surgery in Cipto Mangunkusumo Hospital, Jakarta showed declined percentage during the pandemic.[3] The consequences of this adjustment are still not yet known.

The effect of Covid 19 condition on the decrease in the number of patients and surgical procedures will certainly have an impact on the practice and study of urology. More data needed to show how this situation affects urology practices in one of "type A" hospital in Indonesia which also as a training hospital for residencies. This study was conducted to described the urology practices and residency programs during the Covid-19 era in West Java's referral hospital.

## Methods

This retrospective descriptive study will compare the number of total patients in outpatient, inpatient, and emergency department, and the number of surgical procedures performed in the Urology Department carried out before and during the Covid-19 pandemic.

Sample was obtained from hospital data from the Administrative Department of Dr. Hasan Sadikin General Hospital Bandung. Sample size was collected from all data of surgical procedures performed from January 2019 to December 2020, all data were included in the study.

Type and complexity of the urology cases and surgeries were not included in the sample requirement selection criteria. No questionnaire was made, no interview was conducted. Ethical approval was given by the medical record administrative department. Ethical approval number LB.02.01/X.6.5/74/2022. The study was done in the Urology Department Dr. Hasan Sadikin General Bandung, Indonesia from March to April 2021.

## Result

The result of this current study indicates a decrease in the number of total patients in inpatient, outpatient, and emergency department during the Covid 19 year. This study also showed a decrease in the number of surgery procedures performed at Dr. Hasan Sadikin General Hospital Bandung in 2020 compared to 2019.

Data showed that the total inpatients number in 2019 was 1149, while in 2020 the total inpatients was 678. This data showed a significance decrease of 40.94% in the Covid-19 year compared to the non Covid-19 year. The graph of total inpatient presented in Figure 1. Total outpatient number in 2019 was 7314 and total outpatient number in 2020 was 6722. This data showed a decrease of 7.39% in the Covid-19 year. The graph of total outpatient presented in Figure 1.

The number of patients at emergency department in 2019 was 387, and in 2020 was 262. This study showed 32.30% decrease of the emergency cases in 2020 compared to 2019. The graph of total emergency cases presented

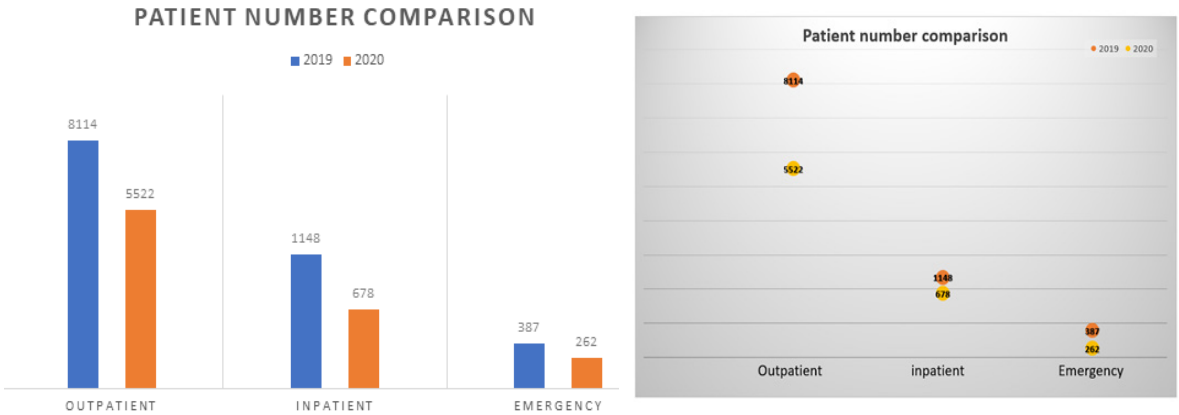


**Figure 1 Comparisons of the Number of Patients in 2019 and 2020**

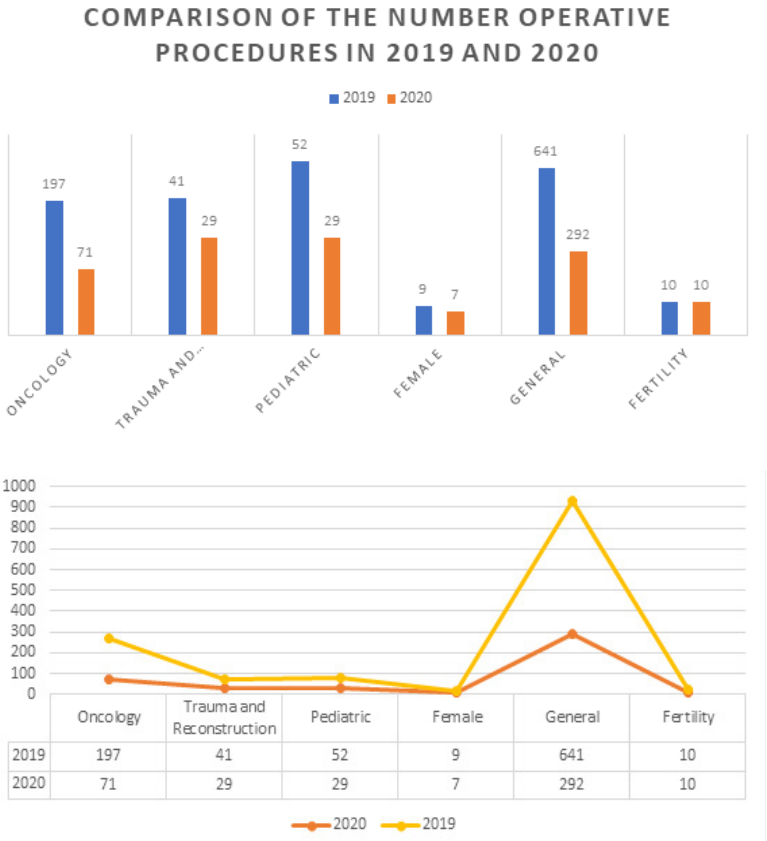


**Figure 2 Comparison of the Number of Operative Procedures in 2019 and 2020**

in Figure 1. Surgical procedures done in 2020 also decreased significantly by 53.89%. The total number of surgical procedures in 2019 was 950 and 438 during the 2020 pandemic. Figure 2 showed the total surgical procedures divide by each division.

This study also compared the number of operations for residents each semester. Our study showed 10th semester residents underwent an average of 150 open surgeries and 398 endoscopic surgeries in 2019. However, in 2020, the average procedure were 71 open surgeries


**Figure 3 Comparison of the Number of Surgeries For Open and Endoscopic in 2019 and 2020**

and 149 endoscopic surgeries. This showed almost 52.6% reduction of open procedures and 62.6% of endoscopic procedures. 9th semester residents underwent an average of 142 open surgeries and 310 endoscopic surgeries in 2019. While in 2020, the average procedure were 56 open surgeries and 217 endoscopic surgeries. This study showed 60.6% reduction of open procedures and 30% reduction of endoscopic procedures. 8th semester residents had an average of 145 open surgeries and 316 endoscopic surgeries in 2019. Meanwhile in 2020 average procedure for each resident were 77 open surgeries and 468 endoscopic surgeries. This showed 46.9% decrease of open procedures, and however 48.1% increase of endoscopic procedures. 7th semester residents had an average of 116 open surgeries and 133 endoscopic surgeries in 2019. While in 2020 average procedure for each resident were 48 open surgeries and 76 endoscopic surgeries. This showed 58.6% decrease of open procedures and 42.8% reduction of endoscopic procedures. The decreased of surgical procedures for residents are presented in Figure 3.

## Discussions

The health system has some aspects affected from the Covid-19 pandemic. Some very important adjustments need to be made to prevent health service providers from this virus infection. To be able to adjust to this pandemic, several recommendations have been made by the health authorities. This includes aspects of daily practice such as outpatient polyclinics, operating rooms, and the use of personal protective equipment (PPE). Some of the recommendations include conducting distance consultations through virtual clinics and postponing non-urgent elective surgeries.[3] A new or revised Hospital regulations are made to deal with the situation. In a teaching hospital, not only the patient health services but also the residents training programmed will be affected.
In urology department, health services include inpatient, outpatient, emergency department, and surgical procedures. Based on our study, all departments experienced a decrease in the number of patients in 2020. Our hospital regulations also took part in this decline phenomenon. In outpatient department, for example, hospital policy was made to prevent crowds in the entrance, admission, and waiting room. These can be reached by reducing the number of patients visits each day. Patient also have to make an online registration before visiting. While in inpatient department, our previous surgical ward was allocated for isolation and treatment of Covid-19 patients. Therefore, the available bed capacity for surgical treatment patients is reduced. Likewise for surgical procedures, operation room allocated for Covid-19 surgical procedure will reduced the available capacity for elective non Covid-19 surgical procedures.

Cases of non-emergency urology or benign disease should be postponed, such as cases of benign prostatic hyperplasia, erectile dysfunction, reconstruction, genitourinary prolapse, and infertility.[3] A present study in Europe displayed a decline of total numbers of surgical procedures during the COVID-19 pandemic, it was significantly lower 53.89% in 2020 compared to 2019.[7]

The latest EAU guidelines has provide some guidance to increased safety on operative procedure. Safety instructions have been given during this pandemic including regarding preoperative management, general surgical procedures, and surgery for patients who are positive for covid 19. According to the existing guidelines, in the preoperative stage, patients who have some clinical symptoms of covid 19 and/or patients who have been in contact with people who are positive for covid 19 must be tested for covid 19 first. Surgical patients who are positive for COVID-19 must have a special operating room prepared. Surgical procedures should be performed by experienced surgeons and anyone who is not involved with the surgical process should not be in the operating room. It is necessary to consider and be careful in performing surgery for elderly patients and patients who have comorbidities.[7]

As it was mentioned before, COVID-19 pandemic has influenced a lot of life aspects, including the residency training programs and medical graduate education. Residents, the frontlines of medical services, might have extra precaution especially proper use of PPE is vital. In many countries, most clinical rounds are canceled.[8] Medical training and learning programs at the hospitals and the medical faculties have changed during the COVID-19 pandemic to become remote or online/virtual activities. In Singapore, the movement of hospital staff is restricted both within and between hospitals. Meanwhile, residents in several hospitals were asked to stay in the hospital without a time limit. However, all of this is being

considered to ensure that this action does not harm all health workers. All the problems that arise as a result of this pandemic such as delays in hospitalization cases, non-emergency elective procedures, and termination of clinical rounds do not result in stress among residents and students.[3]

Based on Abedi et al.,[9] the Iranian residents has restrictions opportunity in both clinical and surgical training activities during pandemics. Significant declined in number of operative procedures that decreased the urological residents to participate in operations per week while performing surgery is the most crucial factor of urological residency education. Elevation of worries in senior residents also reported regarding their operative training. Non-emergency procedures includes endoscopic and open surgery reduce in this pandemic era or it can be postponed to prevent viral transmission. Residency training in important surgical skills for open, endoscopic, laparoscopic, and robotic procedures in this era, virtual reality simulators at hospital and home laparoscopy box trainers have been chosen to maintain residents technical skillset. Instead, these simulations may never be an ideal learning examples for live surgeries.[9]

Changes due to the impact of this pandemic can disrupt urology residency training, this is because patient care is only provided for cases that cannot be deferred. This was also the case in other study that had been conducted, where delays in medical examinations and academic rounds have caused stress among medical students.[10,11] One study in France found that fifty percent of urology residents in training reported experiencing mental stress due to the COVID-19 pandemic. Monitoring and maintaining the health of residents have to be done to prevent their mental fall.[12] Nassar and his colleagues suggest a "Functional Restructuring" of residency training programs during this pandemic so that patient care can still be carried out optimally and while maintaining the mental condition of residents.[13]

In conclusion, the present study showed decreased of total amount patients in inpatient, outpatient, emergency department, and operative procedure during this COVID-19 pandemic. Our study showed decreased of 40.94%, 8.09%, 10,78%, and 53.89% of total inpatients, outpatients, emergency cases, and operative procedure, respectively. This condition might impact in limiting the urological residency exposure to a variety of cases and surgical skill procedures. The residents, department, and hospital should consider ways to minimize the impact on both resident studies and urological services to the community

## References

1. Dashboard data kasus COVID-19 di Indonesia [Internet]. Kementrian Kesehatan Republik Indonesia. [cited 2020 Mar 4]. Available from: https://www.kemkes.go.id/article/view/20031900002/Dashboard-Data-Kasus-COVID-19-di- Indonesia.html.
2. Coronavirus disease (COVID-19) pandemic [Internet]. World Health Organization. 2021 [cited 2020 Mar 4]. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019.
3. Rasyid N, Birowo P, Parikesit D, Rakhman F. The Impact of the COVID-19 Pandemic on Urology Practice in Indonesia: A Nationwide Survey. Urol J. 2020;17(6):677–9.
4. Brücher BLDM, Nigri G, Tinelli A, Lapena Jr JFF, Basany EE, Macri P, et al. COVID-19: Pandemic surgery guidance. 4Open. 2020;3(April):1–46.
5. Amparore D, Claps F, Cacciamani GE, Esperto F, Fiori C, Liguori G, et al. Impact of the COVID-19 pandemic on urology residency training in Italy. Minerva Urol e Nefrol. 2020;72(4):505–9.
6. Ficarra V, Novara G, Abrate A, Bartoletti R, Crestani A, Nunzio CD, et al. Urology practice during the COViD-19 pandemic. Minerva Urol e Nefrol. 2020;72(3):369–75.
7. Ribal MJ, Cornford P, Briganti A, Knoll T, Gravas S, Babjuk M, et al. European Association of Urology Guidelines Office Rapid Reaction Group: An Organisation-wide Collaborative Effort to Adapt the European Association of Urology Guidelines Recommendations to the Coronavirus Disease 2019 Era. Eur Assoc Urol. 2020.
8. Jin P, Park H, Jung S, Kim J. Challenges in Urology during the COVID-19 Pandemic. Urol Int. 2021;105(1–2):163–4.
9. Abedi AR, Aliakbari F, Ghiasy S, Allameh F, Ghanbari MA. The impact of the first covid 19 pandemic on urology residency training in Iran. 2020;4(1):19–22.
10. Thapa BB, Shrestha D, Bista S, Thapa S, Niranjan V. Urology during COVID-19 Pandemic crisis: a systematic review. Surg J. 2021;07(01):e3–10.
11. Khusid JA, Weinstein CS, Becerra AZ, Kashani M, Robins DJ, Fink LE, et al. Well-being and education of urology residents during the

COVID-19 pandemic: results of an american national survey. Int J Clin Pract. 2020;74:1–8.
12. Kwon YS, Tabakin AL, Patel HV, Backstrand JR, Jang TL, Kim IY, et al. Adapting urology residency training in the COVID-19 era. Urology. 2020;141:15–9.
13. Abdessater M, Rouprêt M, Misrai V, Matillon X, Gondran-Tellier B, Freton L, et al. COVID19 pandemic impacts on anxiety of French urologist in training: outcomes from a national survey. Prog Urol. 2020;30(8-9):448–55.

RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v56.3086
Majalah Kedokteran Bandung. 2024;56(1):48–51

Majalah Kedokteran Bandung (MKB)

Received: November 9, 2022
Accepted: March 24, 2023
Available online: March 31, 2024

# Lactic Acid Level as A Predictor of Severity in Patients with Acute Appendicitis

**Raka Aditya,[1] Reno Rudiman,[2] Putie Hapsari[3]**

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

[2]Division of Digestive Surgery, Department of General Surgery, Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia

[3]Division of Vascular Surgery, Department of General Surgery Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia

**Abstract**

Perforated appendicitis is the leading cause of morbidity and mortality of all appendicitis cases in adults and children, with delayed preoperative diagnosis as the main reason. In previous studies, diagnostic modalities such as radiological examination and the current scoring system have been demonstrated as unable to predict the onset of perforated appendicitis. Serological biomarkers of lactic acid are associated with intestinal obstruction and ischemia. The serological value of lactic acid in identifying perforated appendicitis compared to acute one was shown to increase significantly by 0.25 mmol/L ($p<0.05$) in a previous study. This study aimed to determine the correlation between lactic acid and the severity of appendicitis. This was a cross-sectional prospective analytic observational study in patients treated in Dr. Hasan Sadikin General Hospital, Bandung, Indonesia. Subjects were adult patients diagnosed with appendicitis during the period of 2021 in the Emergency Room of the hospital. Data were analyzed using the bivariate analysis and correlation test of difference. This study involved 54 subjects, divided into the complicated appendicitis (study) and control groups, with a mean of lactic acid level of 2,5093 mmol/L (0.9 mmol/L–11.8 mmol/L). In the complicated appendicitis group, 20 subjects (37%) demonstrated an increase in lactic acid level (OR 1.07; 95% CI: -0.03–0.22; p=0.14). The correlation analysis resulted in a negative correlation. This study concluded that there is no significant correlation between lactic acid levels and the severity of appendicitis in these patients.

**Keywords:** Correlation, lactic acid, perforated appendicitis

## Introduction

Acute appendicitis is a frequent case that often found in the field of digestive surgery. Research revealed the incidence of perforated appendicitis in adults ranges from 4–19%. The risk of perforation is very high in the first 24 hours and increases to 6% in the next 36 hours from the beginning of symptoms. Based on this research, surgery is recommended in the first 36 hours of symptoms to prevent complications from appendicitis. Delay in diagnosis is the main cause of these complications. Accurate examination that is able to predict the risk of perforated appendicitis is currently still not found.[1,2]

A complete history taking and good physical examination in cases of appendicitis are believed to have the same accuracy as other investigation modalities in establishing the diagnosis. However, in different cases of complicated appendicitis that cannot be distinguished with certainty from non-complicated appendicitis at the time of the initial examination at the hospital, causing a delay in diagnosis. This problem increases the morbidity and mortality due to the risk of perforated appendicitis.[3]

Several previous studies tried to establish the diagnosis of appendicitis with radiological examinations: CT scan, USG and scoring systems such as the Alvarado score, PAS (Pediatric Appendicitis Score) and AlR (Appendicitis Inflammatory Response). These supporting modalities have proven to be very useful in predicting acute appendicitis in patients with

**Corresponding Author:**
Raka Aditya,
Department of General Surgery, Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia,
Email: rakaaditya0304@gmail.com

complaints of right lower abdominal pain but are still unable to predict the risk of perforated appendicitis. [4,5,6]

Serological biomarker of lactic acid value is associated with the presence of intestinal obstruction and ischemia based on the pathophysiology of appendicitis so that it can facilitate early detection of the risk of perforated appendicitis. Previous studies in patients with perforated appendicitis found higher plasma levels of lactic acid than in patients with acute nonperforated appendicitis (96% sensitivity; 87% specificity).[7] Research in Indonesia on lactic acid biomarkers as a risk predictor of perforated appendicitis is rare and currently have not been done in Bandung. The researcher intends to find out the correlation between lactic acid values as a predictor of perforated appendicitis in adult patients who had been previously diagnosed with acute appendicitis in Dr. Hasan Sadikin General Hospital. This research is expected to be able to provide benefits for clinicians to conduct early intervention in cases of appendicitis at risk of perforation with appropriate and efficient investigations.

## Methods

The study was conducted on subjects who met the inclusion criteria, adult patients (>18 years), diagnosed with appendicitis which was established through history taking, physical examination, Alvarado and Tzanakis scoring, underwent emergency surgery and did not meet the exclusion criteria. The research was conducted at Dr. Hasan Sadikin General Hospital in the period from January 1st 2021 to June 1st 2021. The research was carried out after obtaining Ethical Approval No: LB.02.01//X.6.5/343/2021 from Health Research Ethics Committee of Dr. Hasan Sadikin General Hospital.

This study is a prospective analytic observational study with a cross-sectional design. The independent variable is the value of blood lactic acid and the dependent variable is perforated appendicitis. The sample size is determined by the two-proportion hypothesis formula with each proportion totaling a minimum of 66 samples. The sampling method is by consecutive sampling.

Data processing through the steps of editing, coding, data entry and cleaning. Analysis of the characteristics data was analyzed descriptively. Bivariate analysis uses simple logistic regression bivariate analysis. The risk probability in bivariate analysis is shown as crude odds ratio (OR) with 95% confidence interval (CI). The p value is considered significant if $p < 0.05$. The OR value is considered a risk factor if the OR ≥1.00. The analysis between numerical variables and categorical variables uses the independent T test if the data is normally distributed and the Mann Whitney test if the data is not normally distributed. The normality test of the data used the Kolmogorov Smirnov test. The candidate variables that will be included in the multivariate analysis are variables that have a p value<0.25. The multivariate analysis uses multiple logistic regression with a determinant model. Statistical analysis was processed with SPSS version 21.0 for Windows.

## Results

This study involved 54 subjects in two groups, consisted of the acute appendicitis group (as the study group) and the complicated appendicitis group (as the control group) by monitoring the increase in lactic acid in these patient groups (Table 1). At the characteristics of the subjects, It was found that the subjects suffering from appendicitis were more women than men with a ratio of 1.25:1 with a mean value of age was 40.16 years old (18–81). In the characteristic description based on the severity of the disease, it was found that 94.4% perforated appendicitis and 5.6% acute appendicitis (Table 1). Based on the increase in lactic acid, it was found that 37% (20 subjects) had average value of lactic acid at

**Table 1 Characteristics of The Subjects**

| Variables | n=54 |
|---|---|
| Age | |
| Mean±Std | 40.16±15.5 |
| Range (min-max) | 18,00-71,00 |
| Sex | |
| Male | 24(44.4%) |
| Female | 30(53.6%) |
| Severity | |
| Complicated Appendicitis | 51(94.4%) |
| Acute Appendicitis | 3(5.6%) |
| Level of Lactic Acid | |
| Increase | 20(37%) |
| Normal | 34(63%) |

**Table 2. Simple Regression Analysis for Variables of Perforated Appendicitis Predictors**

| Variable | Perforated Appendicitis | | Acute Appendicitis | | OR | CI 95% | | P-Value |
|---|---|---|---|---|---|---|---|---|
| | n | % | n | % | | Min | max | |
| Sex | | | | | | | | |
| Male | 24 | 47 | 0 | 0 | | | | |
| Female | 27 | 53 | 3 | 100 | -0,11 | -0,23 | 0,02 | 0,09 |
| Onset of Symptom's | | | | | | | | |
| <24 hours | 0 | 0 | 3 | 100 | 9,86 | 1,99 | 3,01 | 0,00 |
| >24 hours | 51 | 100 | 0 | 0 | | | | |
| Lactic Acid | | | | | | | | |
| Increase | 20 | 39 | 0 | 0 | 1,07 | -0,03 | 0,22 | 0,14 |
| Normal | 31 | 61 | 3 | 100 | | | | |

2.5093 mmol/L (0.9-11.8).

Based on the Simple Regression Logistic test, there was an increase in lactic acid levels in subjects with acute appendicitis and perforated appendicitis with p value>0.05 (p=0.14; OR:1.07; 95%CI: -0.03-0.22) so that there was no significant difference between lactic acid levels in patients with acute appendicitis and perforation. The results were the same for the gender variable with p value > 0.05 (p=0.09; OR: -0.11; 95%CI: -0.23-0.02). Appendix obstruction based on symptom onset with a time limit of 24 hours showed a significance value of p < 0.05 (p = 0.00; OR: 9.86; 95% CI: 1.99-3.01) so it can be concluded that there was a significant difference between lactic acid levels in patients with obstruction less than 24 hours and more than 24 hours (Table 2). In the correlation analysis of the value of lactic acid and the severity of appendicitis, the Pearson Correlation value was 0.186, which means the correlation is very weak (Table 3).

**Table 3 Pearson Correlation Between Lactic Acid and Severity of Appendicitis**

| | | KAL_X | KUB_Y |
|---|---|---|---|
| KAL_X | Pearson Correlation | 1 | **.186** |
| | Sig. (2-tailed) | | .178 |
| | N | 54 | 54 |
| KUB_Y | Pearson Correlation | **.186** | 1 |
| | Sig. (2-tailed) | .178 | |
| | N | 54 | 54 |

## Discussion

In this study, univariate and bivariate analysis was carried out on the characteristics of the research subjects. In this study, there was no significant difference between gender in acute and perforated appendicitis patients. This is in accordance with the results of a study in Turkey involving 576 appendicitis patients which showed that the group of perforated appendicitis was dominated by male sex, but there was no significant difference between men and women in the statistical test of both groups.[7]

In the bivariate analysis between increased levels of lactic acid in patients with acute appendicitis and perforation, the p value> 0.05 (p = 0.14; OR:1.07; 95%CI: -0.03-0.22) It means that were no significant results between the increase in the value of lactic acid with the severity of appendicitis. These results were strengthened by the correlation analysis of lactic acid values and the severity of appendicitis, the Pearson Correlation value was 0.186, which means the correlation was very weak. This was probably because patients with high lactic acid levels and unstable hemodynamics parameters who were admitted to emergency department in Dr. Hasan Sadikin General Hospital, had already received intensive fluid intravenous therapy earlier based on sepsis bundle protocols and were proved by intraoperative findings where the complications of appendicitis do not occur. This assumption is in accordance with studies conducted by previous studies that patients who received intravenous fluid therapy more than 30 minutes since the diagnosis of sepsis was made had a higher mean lactic acid level (3 mmol/L) than patients who received intravenous fluids before

30 minutes the diagnosis of sepsis was made (2.6 mmol/L). The result was that patients who received intravenous fluids before 30 minutes had a 12% shorter length of stay compared to patients who received intravenous fluids after 30 minutes (HR 1.14; 95% CI, 1.02-1.27).[4,8,9]

This study has several limitations that might have an influence on the results of this study which showed a negative correlation between lactic acid levels and the incidence of perforated appendicitis. This study had a sample size that was less than the minimum number of samples because the study was conducted in a type A general hospital (main refferal hospital) where cases of acute appendicitis were rare. The research was only conducted in one hospital, so the amount of research data was still limited. Most of the patient's lactic acid data was not taken every day so the researcher must adjust the clinical data with the lactic acid data on the day when the patients were admitted to the hospital.

The conclusion of this study was that there was no significant correlation between lactic acid levels and the severity of appendicitis in Dr. Hasan Sadikin General Hospital Bandung. This study could be used as a clinician's/ surgeons consideration to choose the laboratory parameters for supporting the diagnosis of severity of appendicitis. Surgeons are expected not to rely solely on the lactic acid parameter because it could be proven that it had no correlation with the severity of appendicitis based on this study. Further research is needed by involving a larger number subjects in multicenter and using multivariable parameters which will be tested for statistical quality as a predictor of severity in appendicitis patients.

## References

1. Chambers D, Huang C, Matthews G. Schwartz's Principals of Surgery.11th ed. New York; McGraw Hill; 2019. p.1241–59.
2. Ferris M, Quan S, Kaplan BS, Molodecky N, Ball CG, Chernoff GW.et al. The global incidence of appendicitis. Ann Surg. 2017; 266(2):237–41.
3. Nazir MS, Parveen N, Anjum S, Nadeem N, Sohail H, Sohail M.et al. Diagnostic accuracy of lactate and c-reactive protein in patients with acute appendicitis. Pakistan J Med Heal Sci. 2020; 14(3):540–2.
4. Kim TH, Cho BS, Jung JH, Lee MS, Jang JH, Kim CN. Predictive factors to distinguish between patients with noncomplicated appendicitis and those with complicated appendicitis. Ann Coloproctol. 2015;31(5):192–7.
5. Montagnana M, Danese E, Lippi G. Biochemical markers of acute intestinal ischemia: possibilities and limitations. Ann Transl Med. 2018;6(17):341.
6. Shi H, Wu B, Wan J, Liu W, Su B. The role of serum intestinal fatty acid binding protein levels and D-lactate levels in the diagnosis of acute intestinal ischemia. Clin Res Hepatol Gastroenterol. 2015. 39(3):373–8
7. Afaque MY, Rehman N, Rizvi SAA, Ahmed M. Early Recognition and Management of Small Bowel Perforation. In: Goenka M, Goenka U, Rodge GA, eds. Endoscopy in Small Bowel Diseases. Intech Open. 2021. 3(8):1–8.
8. Nazir MS, Parveen N, Anjum S, Nadeem N, Sohail H, Sohail M, Sulehri AA. Diagnostic Accuracy of Lactate and C-reactive Protein in patients with acute appendicitis. Pakistan Journal of Medical & Health Sciences. 2020; 14(3):540–42.
9. Lee J, Byun Y, Park J, Lee J, Ryu J, Choi S. Lactic acid level as an outcome predictor in pediatric patients with intussusception in the emergency department. BMC Pediatrics Journal. 2020; 184(20):1–10.
10. Virmani S, Prabhu PS, Sundeep PT, Kumar V. Role of laboratory markers in predicting severity of acute appendicitis. African J Paediatr Surg. 2018;15(1):1–4.

RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v56.3070
Majalah Kedokteran Bandung. 2024;56(1):52–57

Majalah Kedokteran Bandung (MKB)

Received: October 25, 2022
Accepted: February 16, 2023
Available online: March 31, 2024

# Vitamin D Levels and Incidence of Preterm Labor

**Muhammad Alamsyah Aziz, Chandra Garnida Syahbana, Jusuf Sulaeman Effendi, Benny Hasan Purwara, Tita Husnitawati Madjid, Hadi Susiarno**

Department of Obstetrics and Gynecology, Faculty of Medicine Universitas Padjadjaran
Dr. Hasan Sadikin General Hospital Bandung, Indonesia

**Abstract**

One pathophysiology of preterm delivery is maternal or fetal hypothalamus-pituitary-adrenal (HPA) axis activation. The HPA axis can be affected by vitamin D, which increases uterine contractions and affects the body's immune mechanism against bacterial infections. A lower level of vitamin D in pregnant women is suspected to contribute to the incidence of premature conditions. This study aimed to compare the 25-hydroxy-vitamin D3 concentration in preterm parturient with non-preterm parturient, and the correlation between vitamin D level and the incidence of preterm labor. This comparative analytic study used a cross-sectional approach and involved 46 subjects who were divided into case and control groups. This study was conducted in August–September 2017 at Dr. Hasan Sadikin General Hospital, Bandung, Indonesia. The Electro-chemiluminescence Immunoassay (ECLIA) method was used to examine the serum 25-hydroxy-vitamin D3 concentration in case and control groups, demonstrating that 25-hydroxy-vitamin D3 concentration in case group (17.26 ng/mL) was significantly ($p<0.0001$) lower than in control group (24.30 ng/mL). The correlation coefficient between the 25-hydroxy-vitamin D3 and the incidence of preterm labor was -0.837 ($p<0.001$). Thus, there was a correlation between the 25-hydroxy-Vitamin D3 level and the incidence of preterm labor that vitamin D supplementation in pregnant women must be considered.

**Keywords:** 25-hydroxy-vitamin D3, preterm delivery, vitamin D

## Introduction

Vitamin D has roles in different organ systems.[1] Vitamin D is one of the nutrients needed during pregnancy that has a role in maternal and fetal calcium metabolism and bone health.[2] In many studies, vitamin D was suggested to have a role in various body systems, such as the nervous system, immune system, circulation system, and regulation of expression of specific genes.[3-6]

In the past several years, the prevalence of vitamin D deficiency and the incidence of low vitamin D status have increased in developing and some developed countries.[7] Several reports indicate that vitamin D deficiency has a role in the pathophysiology of cancer, cardiovascular disease, diabetes, and morbidity in pregnancy.[2] Vitamin D statuses got special attention related to exposure to ultraviolet rays in Southeast Asian countries. Singapore is one of the countries where the population matches the research on the association between vitamin D status and UV exposure. Singapore has a slightly lower prevalence of vitamin D deficiency than Thailand, possibly related to the geographical site closer to the equator.[1] Previous cohort study by Jusdistiani et al.,[8] at four cities in West Java, showed that around 195 pregnant women (96.06%) had hypovitaminosis D (< 30 ng/mL), which approximately 152 (75%) had vitamin D deficiency, and 43 (21%) had vitamin D insufficiency. Most who had insufficiency also had anemia.

Vitamin D deficiency, the level in serum < 20 ng/mL, is known as the cause of osteoporosis, muscle impairment, pain, and fractures. That was a significant public health problem.[1] Maternal vitamin D deficiency during pregnancy occurs commonly in developed countries.[9] However, vitamin D deficiency in pregnancy

**Corresponding Author:**
Muhammad Alamsyah Aziz
Department of Obstetrics and Gynecology, Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia
Email: alamsyahaziz9119@gmail.com

cause disorders in pregnancy, including preterm labor and adverse neonatal outcomes.[10]

Vitamin D was known to have a role in preterm delivery.[11] It can affect the hypothalamus-pituitary-adrenal (HPA) axis by increasing uterine contractions and affecting the body's immune mechanism against bacterial infections. This study will prove that the incidence of preterm birth has occurred due to vitamin D deficiency. So, this study aims to analyze and compare the relationship of vitamin D levels between the incidence of preterm labor in preterm and non-preterm parturient patients.

## Methods

The research method used was a cross-sectional comparison analysis. The research materials were obtained from the medical records of Dr. Hasan Sadikin General Hospital from August–September 2017. This study took blood samples from preterm labor patients with 2834 weeks gestation age ranges and the results were 46 subjects consisting of 23 subjects of preterm parturient patients (cases) and 23 subjects of non-parturient preterm (controls). This study analyzes the correlation between research variables. Descriptive analysis is used to see the frequency distribution and percentage of various independent or dependent research variables. Statistical analysis used to analyze patient category characteristic data was tested by chi-square test and unpaired T. The relationship between preterm labor and vitamin D levels was tested with the Mann-Whitney test. The significance criteria used is p-value ≤0.05 as significant or statistically significant, and p-value >0.05 as not significant or not statistically significant. The Biserial Point test tested the 25-hydroxy-Vitamin D3 levels and the incidence of preterm labor correlation. The SPSS Program version 23.0 for Windows was used to conduct the statistical tests. All subjects provided written informed consent for participating the study. This study was approved by the Research Ethics Committee RSUP Dr. Hasan Sadikin Hospital Bandung with registered number LB.04.01/A05/EC/228/VIII/2017.

## Results

During this study period, 46 subjects consisting of 23 subjects of preterm parturient patients (cases) and 23 subjects of non-parturient preterm (controls) occupied the inclusion and exclusion criteria. The 23 subjects of preterm

**Table 1 Subject Characteristics**

| Variable | Group | | p-value |
|---|---|---|---|
| | Case | Control | |
| | n=23 | n=23 | |
| Age (year) | | | |
| Mean±STD | 28.35±2.85 | 27.96±4.40 | 0.722* |
| Median | 28.00 | 28.00 | |
| Range (min-max) | 22.00–34.00 | 18.00–35.00 | |
| BMI (kg/m$^2$) | | | |
| Mean±STD | 22.64±1.45 | 22.71±1.72 | 0.884* |
| Median | 22.97 | 23.11 | |
| Range (min-max) | 19.72–25.39 | 19.14–25.39 | |
| Parity | | | |
| Nulliparous | 8 (34.80%) | 11 (47.80%) | 0.869** |
| Multiparous | 15 (65.20%) | 12 (52.20%) | |
| Occupation | | | |
| Civil servant/private (working) | 11 (47.80%) | 10 (43.50%) | 0.913** |
| Housewives (not working) | 12 (52.20%) | 13 (56.50%) | |

Description: * based on unpaired T test; ** based on chi square test

**Table 2 Correlation between 25-hydroxy-vitamin $D_3$ Levels in Preterm Parturient and Non-Preterm Parturient Groups**

| Variable | Group | | p-value |
|---|---|---|---|
| | Case | Control | |
| | n= 23 | n= 23 | |
| Vitamin D (ng/mL) | | | |
| Median | 17.26 (1.6) | 24.30 (3.0) | <0.0001* |
| Range (min-max) | 15–21 | 18–31 | |

Description: * based on unpaired T test

parturient patients were hospitalized at Dr. Hasan Sadikin General Hospital, and the 23 subjects of non-preterm parturient patients (control) were treated at Obstetrics Clinic Dr. Hasan Sadikin General Hospital who had homogeneous characteristics with preterm pregnancy subjects. The blood sample from every subject in this study has been taken to analyze the levels of 25-hydroxy-vitamin D3.

Table 1 shows the case and control subjects characteristics based on age, parity, body mass index (BMI), and occupation. The mean age was 28.15±3.67 years, and the mean BMI was 22.68 ±1.58 kg/$m^2$. It can be seen that nulliparous parity was 19 (41.30%), and multiparous parity was 27 (58.70%). The 21 people (45.70%) were civil servants/private employees (working), and 25 people (54.30%) were housewives (not working).

Table 1 compares the age, BMI, and occupation of preterm parturients (case group) and non-preterm parturients (control group). The patients' mean age in the case group was 28.35±2.85 years, and the control group was 27.96±4.40 years. The mean BMI in the case group was 22.64±1.45 kg/$m^2$, and in the control group was 22.71±1.72 kg/$m^2$. The number of civil servants/private employees in the case group was 11 people (47.80%), and the number of housewives was 12 people (52.20%), while in the control group, only 10 people (43.40%) were working people and 13 people (56.50%) were not working people. Nulliparous parity in the case group was eight people (34.80%), and multiparous was 15 people (65.20%), while in the control group, nulliparous was 11 people (47.80%), and multiparous was 12 people (52.20%).

Numerical data, such as age and BMI, normally distributed data, have been tested using an unpaired T-test. The result showed that all of this study's variables were insignificant (p>0.05). It can be explained that there was no significant difference in age and BMI variables between the case and control groups.

Categorical data, such as parity and occupation, were analyzed using Chi-Square. However, only occupation data can be tested by the Chi-Square test. In contrast, the parity data was tested by an alternative test, the Exact Fisher test, because the Chi-Square test's requirement was not fulfilled. The result showed no significant or statistically significant difference (p>0.05) between the parity and occupation variables in the two study groups: the preterm parturient and the non-preterm parturient.

Based on the analysis of the patient characteristics, all p-value >0.05 showed that at the beginning of the examination, both groups were equal or had no characteristic difference. This indicates that both groups are homogeneous, which means all of the characteristics fulfilled the requirements to compare and test for further statistical hypotheses.

The 25-hydroxy-Vitamin D3 levels in the serum of both groups were described in Table 2. It was shown that the serum 25-hydroxy-Vitamin D3 concentration in the control and case groups was 17.26 ng/mL and 24.30 ng/mL, respectively. The result showed a statistically significant difference (p<0.0001, using the Mann-Whitney test) between the mean value of variables 25-hydroxy-vitamin D3 in the case and control groups.

Correlation coefficient (r) analysis between 25-hydroxy-vitamin $D_3$ levels and the incidence of preterm labor value was -0.837 with p-value >0.001 (based on Biserial Point Correlation Coefficient). It showed that significant correlation or statistically significant. Thus, it can be concluded that there was a correlation between 25-hydroxy-Vitamin D3 levels and the incidence of preterm labor.

## Discussion

In this study, the characteristics of the research subjects compared were age, BMI, parity, and occupation because these four factors are suspected to be confounding factors in the results of this study. The characteristics of the subjects between the two groups must be homogeneous. Thus, the two groups could be compared to each other. The results showed that there was no significant difference in all of the characteristics of this study subjects, which are age (p-value= 0.201), BMI (p-value=0.884), parity (p-value =0.369) and occupation (p-value=0.913). It means that both groups are homogenous. Therefore, the bias between age, BMI, parity, and occupation in both groups could be diminished so that both groups could be compared.

This study showed that the 25-hydroxy-vitamin D3 level in preterm parturient patients was significantly lower than in non-preterm parturient patients. Around 47.82% of subjects had 25-hydroxy-vitamin D3 levels lower than 20 ng/mL, which means the subjects sustained deficiency. About 52.18% of subjects had been included in normal groups (≥ 20 ng/mL). Vitamin D deficiency is a global health problem. At least 20 ng/mL of 25-hydroxy-vitamin D3 levels are needed for bone health.[12] In this study, nearly 91.30% of preterm parturient subjects sustained vitamin D deficiency, and the rest were normal. In the preterm non-preterm parturient subjects, 4.35% of it had been included in the deficiency group and 95.65% in the normal group.

Vitamin D is essential in maintaining calcium homeostasis, bone development, and mineralization. The primary source of vitamin D was exposure to ultraviolet B waves (290–320 nm).[13] The previous observational study described that vitamin D deficiency became one of the factors in the pathophysiology of some cancer, cardiovascular disease, hypertension, diabetes, and obstetrical morbidity. In 2005–2006, 69% of Indonesian pregnant women and 78% of non-pregnant women had 25-hydroxy-vitamin D3 levels lower than 30 nmol/L based on National Health and Nutrition Examination Survey (NHANES).[14]

In both groups, the distribution results of 25-hydroxy-vitamin D3 level and age showed that the control group was spread over the age range 18 to 35 years old, have the mean value of 25-hydroxy-vitamin D3 level being 24.30 ng/mL, whereas in the case group was spread over the age range 22 to 34 years old have the mean of 17.26 ng/mL. Preterm pregnancy, which was previously unknown, is now being discovered through several studies. The NHANES study found that preterm pregnancy occurred at various ages. Approximately 82% of pregnant women had vitamin D deficiency, which is 34% of them happened at age above 35 years old, 14% occurred above 40 years old, and the rest happened at 45 years old. Women who have a BMI above the normal range are found that they have vitamin D deficiency.[15]

Based on the results that have been presented, it can be concluded that there was a correlation between the 25-hydroxy-vitamin D3 level and the incidence of preterm labor. Statistical analysis results of the correlation of the 25-hydroxy-vitamin D3 and the incidence of preterm labor, which was tested by the biserial point correlation, showed that r=-0.837 with p-value <0.001. This result is accorded with the study which had done by Holick et al.,[10] which stated an inverse correlation between the 25-hydroxy-vitamin D3 and the incidence of preterm labor. Liu et al.[11] conducted a cohort of 39 pregnant women with preterm labor, which explained a statistically significant result (p = 0.002) that the lower the vitamin D level, the higher the incidence of preterm labor.

Vitamin D supplementation is known to decrease the body's response to pathogens by reducing the production of interleukin-6, interleukin-1, and tumor necrosis factor compounds by macrophages, thus playing a direct role in regulating myometrial contractility.[16,17] Vitamin D is also known to directly affect myometrial contractility by regulating Connexin-43 gene expression (gap junction association protein) at the transcriptional level of mRNA through nuclear VDR.[18] Study also suggested that vitamin D was able to reduce cytokines and Contractile-Associated Protein (CAP) due to the induction of inflammation in human myometrial smooth muscle cells and could prevent preterm birth through inhibition of infection/inflammatory pathways.[19,20]

The correlation between vitamin D and the incidence of preterm birth in the Indonesian population still needs further research. However, this has proven that preterm birth is associated with maternal vitamin D deficiency status. Thus, it can be concluded that the effectiveness of vitamin D as an alternative non-surgical and non-invasive therapy in cases of preterm labor requires further research. Vitamin D supplementation during pregnancy must be considered.

The limitation of this study is that it does not

exclude confounding factors such as age, BMI, parity, and occupation. Further research can be done on more homogeneous subjects.

Based on the result of this study, it is concluded that there was a significant difference between 25-hydroxy-vitamin D3 levels in preterm parturient patients and non-preterm parturient patients. There was also a correlation between the 25-hydroxy-vitamin D3 and the incidence of preterm labor. Dietary recall of meals containing vitamin D and sun ray exposure must be further assessed in the following study. Vitamin D supplementation in pregnant women must be considered.

## References


1. Nimitphong H, Holick MF. Vitamin D status and sun exposure in southeast Asia. Dermatoendocrinol. 2013;5(1):34–7. doi:10.4161/derm.24054
2. Wagner CL, Taylor SN, Johnson DD, Hollis BW. The role of vitamin D in pregnancy and lactation:emerging concepts. Womens Health (Lond). 2012;8(3):323–40.
3. Christakos S, Hewison M, Gardner DG, Wagner CL, Sergeev IN, Rutten E, et al. Vitamin D: beyond bone. Ann N Y Acad Sci. 2013;1287(1):45–58. doi:10.1111/nyas.12129
4. Rosen CJ, Adam JS, Bikle DD, Black DM, Demay MB, Manson JE, et al. The nonskeletal effects of vitamin D: an Endocrine Society Scientific Statement. Endocrine Rev. 2012; 33(3):456–92.
5. Gil A, Plaza-Diaz J, Mesa M, D: Vitamin D: Classic and Novel Actions. Ann Nutr Metab 2018;72:87–95.
6. Gernand AD, Klebanoff MA, Simhan HN, Bodnar LM. Maternal vitamin D status, prolonged labour, cesarean delivery, and instrumental delivery in an era with a low cesarean rate. J Perinatol. 2015;35:23–8.
7. Bener A, Al-Hamaq A, Saleh NM. Association between vitamin D insufficiency and adverse pregnancy outcome: global comparisons. Int J Womens Health. 2013;5:523–31.
8. Judistiani RTD, Madjid TH, Irianti S, Natalia YA, Indrati AR, Ghozali M, Sribudiani Y, et al. Associated of first trimester maternal vitamin D, ferritin and hemoglobin level with third trimester fetal biometry: result from cohort study on vitamin D status and its impact during pregnancy and childhood in Indonesia. BMC Pregnancy Childbirth. 2019; 19(112):1–8.
9. Ioannou C, Javaid M, Mahon P, Yaqub M, Harvey N, Godfrey K, et al. The effect of maternal vitamin D concentration on fetal bone. J Clinical Endocrin Metabolism. 2012;97(11):E2070–E7.
10. Calton EK, Keane KN, Newsholme P, Soares MJ. The impact of Vitamin D levels on inflammatory status: a systematic review of immune cell studies. PLoS One. 2015;10(11):e0141770. doi:10.1371/journal.pone.0141770
11. Liu N, Kaplan A, Low J, Nguyen L, Liu G, Equils O, et al. Vitamin D induces innate antibacterial responses in human trophoblasts via an intracrine pathway 1. Biol Repro. 2009;80(3):398–406.
12. De-Regil LM, Palacios C, Lombardo LK, Peña-Rosas JP. Vitamin D supplementation for women during pregnancy. Cochrane Database Syst Rev. 2016;(1):CD008873. doi:10.1002/14651858.CD008873.pub3.
13. Hamilton SA, Mullan C. Management of preterm labour. Obstet Gynaec Repro Med. 2016; 26(1):12–9.
14. Ginde AA, Sullivan AF, Mansbach JM, Camargo CA Jr. Vitamin D insufficiency in pregnant and nonpregnant women of childbearing age in the United States. Am J Obstet Gynecol. 2010;202(5):436.e1-436.e4368. doi:10.1016/j.ajog.2009.11.036
15. Pludowski P, Holick MF, Pilz S, Wagner CL, Hollis BW, Grant WB, et al. Vitamin D effects on musculoskeletal health, immunity, autoimmunity, cardiovascular disease, cancer, fertility, pregnancy, dementia and mortality—a review of recent evidence. Autoimmunity Rev. 2013;12(10):976–89.
16. Schröder-Heurich B, Springer CJP, von Versen-Höynck F. Vitamin D effects on the immune system from periconception through pregnancy. Nutrients. 2020;12(5):1432. doi:10.3390/nu12051432
17. Tehrani FR, Behboudi-Gandevani S. Vitamin D and human reproduction [Internet]. A critical evaluation of vitamin D-basic overview. InTech; 2017. Available from: http://dx.doi.org/10.5772/67394.
18. Leimert KB, Xu W, Princ MM, Chemtob S, Olson DM. Inflammatory amplification: a central tenet of uterine transition for labor. Front Cell Infect Microbiol. 2021;11:660983. doi:10.3389/fcimb.2021.660983.
19. Daneshkhah A, Agrawal V, Eshein A, Subramanian H, Roy HK, Backman V. Evidence for possible association of

vitamin D status with cytokine storm and unregulated inflammation in COVID-19 patients [published correction appears in Aging Clin Exp Res. 2023 Dec;35(12):3263]. Aging Clin Exp Res. 2020;32(10):2141–58. doi:10.1007/s40520-020-01677-y

20. Thota C, Laknaur A, Farmer T, Ladson G, Al-Hendy A, Ismail N. Vitamin D regulates contractile profile in human uterine myometrial cells via NF-κB pathway. Am J Obstet Gynecol. 2014;210(4):347.e1-347.e10. doi:10.1016/j.ajog.2013.11.027

RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v56.3042
Majalah Kedokteran Bandung. 2024;56(1):58–61

Majalah Kedokteran Bandung (MKB)

Received: September 29, 2022
Accepted: May 25, 2023
Available online: March 31, 2024

# Correlation between Duration of Placement of Non-tunneled Hemodialysis Catheter and Positive Bacterial Culture Incidence in Hemodialysis Patients

**Teguh Marfen Djajakusumah,[1] Putie Hapsari,[1] Indra Prasetya Yarman,[1] Hafidh Seno Radi Utomo,[1] Kiki Lukman,[2] Dian Sulastri,[2] Muhammad Faiz Ulurrosyad[2]**

[1]Division of Vascular Surgery, Department of Surgery Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung

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

**Abstract**

Non-tunneling hemodialysis catheter is one of the most convenient vascular accesses for hemodialysis in End Stage Kidney Disease (ESRD) patients. However, these catheters have the highest morbidity among all available accesses. Current guidelines recommend that non-tunneled hemodialysis catheters be placed for no more than 2 weeks to prevent bacterial contamination. This study was conducted in Dr. Hasan Sadikin General Hospital, Bandung, Indonesia, throughout January-December 2021. A correlation test with a prospective observational analysis design was applied to find the link between the duration of non-tunneled hemodialysis catheter placement and the incidence of positive bacterial culture on the catheter. Pearson correlation test was used to see the strength of the correlation, with a significance limit of 0.05. This study involved 28 subjects. The most common location for catheter placement was in the jugular vein, and laboratory examinations showed no correlation between leukocyte values and positive bacterial culture results. Hypertension and diabetes mellitus were not significant risk factors (p-value 0.887). At ≤14 days of catheter placement, only 1 (6.7%) of the subjects presented a positive culture result. Most of the positive bacterial culture results were found at a duration of 15-30 days of placement, (n=8; 53.3%). The results of the Pearson test showed a p-value of 0.036 and an r-value of 0.399, indicating a significant result with a strong correlation between the two variables. The duration of placement of hemodialysis catheters has a positive correlation with the incidence of positive bacterial cultures, which can increase the risk of systemic infection associated with hemodialysis catheters.

**Keywords:** Bacterial culture, correlation, duration of placement, non-tunneled hemodialysis catheters

## Introduction

End-stage renal disease (ESRD) is an irreversible condition that is becoming an ever-growing problem in terms of morbidity, mortality, and cost. The inability of the patient's kidneys to perform their proper function requires the patient to undergo kidney replacement therapy as a means of increasing their life expectancy.[1,2,3]

Dialysis catheters are the most convenient vascular access for hemodialysis as it can be done in multiple places, can be used immediately, are relatively low-cost, are easy to apply and replaced, and do not require venous cannulation to be used for dialysis. Although their benefit also comes with the cost of higher morbidity rate due to the risk of thrombosis and infection.[4]

Bacterial contamination may be predisposed by several factors such as comorbid conditions, method of application, location of placement, and duration of the placement. Several coexisting conditions such as diabetes mellitus and hypertension can alter the immune system and increase risk of infection. Diabetic and hypertensive patients may experience microvascular complications that increase the skin susceptibility to damage. Added with poor blood flow, inhibiting the immune system to take effect in the lesion.[5,6] Non-tunneled catheters are recommended to be used only for up to 2 weeks, placement and usage of more than 2 weeks may lead to an increased risk of contamination.

**Corresponding Author:**
Muhammad Faiz Ulurrosyad,
Department of General Surgery, Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia,
Email: fulurrosyad@gmail.com

Contamination of dialysis catheters is mostly caused by epidermal flora such as *Staphylococcus epidermidis* and *Staphylococcus aureus.* Contamination generally occurs through one of four pathways: colonization on the edge of the catheter by epidermal flora, colonization of the catheter lumen, hematogenic spread from other infectious sites, and contamination of the lumen.[7]

This study aimed to discover the correlation between the duration of placement of non-tunneled dialysis catheters with the incidence of positive blood bacterial culture related to catheter placement in Dr. Hasan Sadikin General Hospital Bandung, Indonesia.

## Methods

This was a cross-sectional study. Subjects include all hemodialysis patients applied with non-tunneled dialysis lines in Dr. Hasan Sadikin General Hospital in January-December 2021. The inclusion criteria for the subjects are hemodialysis patients who have undergone non-tunneled dialysis catheters and agreed to comply with the study. Unrelated infection and infected catheter that required reinsertion in a different site are considered as the exclusion criteria. The minimal sample size was determined to be 28 samples. Demographic data such as gender and age were obtained. Comorbidities are also noted, namely diabetes mellitus and hypertension. Blood samples for culture were collected from the catheter. Subjects were then divided into two groups depending on their bacterial culture result and placement of the catheter. The subjects in each group were categorized based on the duration of catheter placement. Pearson correlational analysis was performed to determine the strength of the correlation between the duration of the placement and bacterial growth. Consent has been obtained from patients and the study has passed ethical clearance from the Health Research Ethic Committee of Dr. Hasan Sadikin General Hospital (document number LB.02.01/X.6.5/266/2021).

## Results

A total of 28 samples were collected. The demography of the subject was predominantly male (15 subjects) with an average age of 49.93±14.40. Subjects with negative bacterial culture consisted of 9 male subjects and 6 female subjects (69.2% and 30.8% respectively of all negative culture subjects).

The location of placement of the catheter

**Table 1 Characteristics of Subjects**

| Variables | Total (n=28) | Positive bacterial culture (n=15) | Negative bacterial culture (n=13) | p* |
|---|---|---|---|---|
| Gender | | | | |
| Male | 15 | 6 | 9 | 0.131 |
| Female | 13 | 9 | 4 | |
| Age (year) | | | | |
| Average | 49.93±14.40 | 51.07±11.38 | 49.31±18.35 | 0.662 |
| Median | 48 | 54 | 58 | |
| Range | 21-74 | 23-70 | 21-74 | |
| Location of placement | | | | |
| Internal jugular vein | 22 | 12 (80%) | 10 (77%) | 0.850 |
| Femoral vein | 6 | 3 | 3 (23%) | |
| Comorbidities | | | | |
| Diabetes mellitus | 3 | 3 | 0 (0%) | 0.887 |
| Hypertension | 15 | 5 | 10 (76.9%) | |
| Diabetes mellitus and hypertension | 10 | 7 | 3 (23.1%) | |

* Pearson test

**Table 2 Duration of Catheter Placement and Culture Result**

| Duration of placement | Total n=28 | Positive Bacterial Culture n=15 | Negative Bacterial Culture n=13 |
|---|---|---|---|
| ≤14 days | 4 (14.3%) | 1 (6.7%) | 3 (23.1%) |
| 15–30 days | 17 (60.7%) | 8 (53.3%) | 9 (69.2%) |
| 1–2 months | 6 (21.4%) | 5 (33.7%) | 1 (7.7%) |
| 2–3 months | 1 (3.6%) | 1 (6.7%) | 0 (0%) |

was mostly found in the internal jugular vein (22 subjects; 78.6%). Mapping of comorbidities found that 53.6% of subjects had hypertension, 35.7% had hypertension and diabetes mellitus, and 10.7% had diabetes mellitus only.

Most of the non-tunneled lines were placed for more than 14 days (24 subjects; 85.7%), with the majority of the subjects ranging from 15-30 days (17 subjects; 60.7%).

The correlation between the presence of bacterial colonies and the duration of catheter placement was analyzed using the Pearson test. A significant result was yielded ($p<0.005$) with a strong positive correlation ($r>0$) as shown in Table 3.

## Discussion

Demographic characteristics of the subjects in this study bear an insignificant correlation with the incidence of bacterial infection. Previous studies also displayed inconclusive and varying results in terms of the correlation between gender and the formation of bacterial colonies.[8,9,10]

The location of catheter placement in this study was mostly in the internal jugular vein which may also explain why there are more subjects with positive bacterial culture in the jugular vein group compared to the femoral vein group. Both groups have a similar proportion of positive bacterial culture and a total number of subjects in the respective group. Pearson's test yielded a non-significant result, which is true with previous studies that had different results regarding the correlation between the location of placement and bacterial culture results.[11]

The proportion of subjects with diabetes mellitus that yielded a positive bacterial culture result appeared to be the highest compared to other comorbidities. Diabetes mellitus is known to interfere with infection control and therefore people with diabetes mellitus are more susceptible in developing bacterial colonies in high-risk areas. This explanation held when we observe that the proportion of subjects that had diabetes mellitus & hypertension with positive culture is also higher compared to subjects with hypertension only. However, an analytical test showed that these comorbidities do not have a significant correlation with culture results, suggesting that other factors play a bigger role in the formation of bacterial colonies.[10]

Crosstabulation between the duration of placement and culture result displayed that the length of placement is following positive bacterial culture result. Almost all positive bacterial culture results (93.3%) came from subjects with a duration of placement of more than 14 days. The analytical test also shows a significant result with a strong positive correlation between the duration of placement and culture result. It is also noteworthy that the longer the duration of the placement, the greater the proportion of duration and bacterial culture result, suggesting that the risk of infection becomes greater the longer that the catheter is in place. This finding

**Table 3 Correlation between Duration of Placement and Bacterial Culture**

| Duration of placement | Total n=28 (%) | Positive bacterial culture n=15 (%) | Negative bacterial culture n=13 (%) | p* | r* |
|---|---|---|---|---|---|
| ≤14 days | 4 (14.3%) | 1 (6.7%) | 3 (23.1%) | 0.001 | 0.739 |
| >14 days | 24 (85.7%) | 14 (93.3%) | 10 (76.9%) | | |

** Pearson test*

supports the current existing guidelines from the National Kidney Foundation/Kidney Disease Outcomes Quality Initiative (NKF/KDOQI) that recommend placement of non-tunneled lines of no more than two weeks.[12,13]

Most of the colonies found in the bacterial culture are colonies of *Staphylococcus aureus*. This discovery is consistent in the duration of placement groups and is following the bacteria found in previous studies. According to previous literature, the second most common colony to be found is coagulase-negative *Staphylococci* (CoNS). We understand now that normal skin florae are the most common pathogen of infection in patients with non-tunneled dialysis lines. An evaluation of aseptic procedures and aftercare may shine some more light on the prevention of these infections.[14]

In this study, the authors have found a strong correlation between the duration of placement of a non-tunneled dialysis catheter with the incidence of positive bacterial culture. The longer the duration of the placement, the greater the risk of infection. This study also supports the existing guidelines and recommendations of non-tunneled line usage of no more than two weeks to prevent unnecessary morbidities and cost inefficiencies due to infection.

## References

1. Eknoyan G, Lameire N, Eckardt K, Kasiske B, Wheeler D, Levin A. KDIGO 2012 Clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney Int. 2014;19(1):4477–83.
2. World Kidney Day. Chronic Kidney Disease. [Internet] 2017 [cited 2022 Feburary 14]; Available from: http://www.worldkidneyday.org/faqs/chronic-kidney-disease/.
3. Pusdatin RI. 2017. Situasi Penyakit Ginjal Kronis. Infodatin. Jakarta: Kementrian Kesehatan RI; 2017. p. 1–12.
4. Bream PR. Update on Insertion and Complications of Central Venous Catheters for Hemodialysis. Semin Intervent Radiol. 2016;33(1):31–8.
5. Abu-Ashour W, Twells L, Valcour J, Randell A, Donnan J, Howse P, et al. The association between diabetes mellitus and incident infections: a systematic review and meta-analysis of observational studies. BMJ Open Diabetes Res Care. 2017;5(1):e000336. doi:10.1136/bmjdrc-2016-000336
6. Su S, Chen R, Zhang S, Shu H, Luo J. Immune system changes in those with hypertension when infected with SARS-CoV-2. Cell Immunol. 2022;378:104562. doi:10.1016/j.cellimm.2022.104562
7. Pinto B, Miriunis C, Pellicia F, Morris I, Romach I. Vascular Access, Management, and Closure. Madrid: Imprenta Tomás Hermanos; 2018.
8. Wang K, Wang P, Liang X, Lu X, Liu Z. Epidemiology of haemodialysis catheter complications: a survey of 865 dialysis patients from 14 haemodialysis centres in Henan province in China. BMJ Open. 2015;5:1–13.
9. Martin K, Lorenzo YSP, Leung PYM, Chung S, O'flaherty E, Barker N, et al. Clinical Outcomes and Risk Factors for Tunneled Hemodialysis Catheter-Related Bloodstream Infections. Open Forum Infect Dis. 2020;7(6):ofaa117.
10. Delistefani F, Wallbach M, Müller GA, Koziolek MJ, Grupp C. Risk factors for catheter-related infections in patients receiving permanent dialysis catheter. BMC Nephrol. 2019;20(1):199. Published 2019 May 31. doi:10.1186/s12882-019-1392-0
11. Ali M, Das B, Kumar S, Memon R, Dayu B, Babeeta. Catheter related infection in hemodialysis patients with double lumen catheter. Professional Med J. 2019; 26(8):1278–82. DOI: 10.29309/TPMJ/2019.26.08.3869
12. Lok CE, Huber TS, Lee T, Shenoy S, Yevzlin AS, Abreo K, et al. KDOQI Vascular Access Guideline Work Group. KDOQI clinical practice guideline for vascular access: 2019 update. Am J Kidney Dis. 2020;75(4)(suppl 2):S1–S164.
13. Basri NS, Patrianef P. Infection of double lumen catheter as hemodialysis access. New Ropanasuri J Surg. 2017;2(1):25–8.
14. Kaze FF, Ashuntantang G, Halle MP, Kengne AP. Outcomes of non-tunneled non-cuffed hemodialysis catheters in patients on chronic hemodialysis in a resource limited Sub-Saharan Africa Setting. Ther Apher Dial. 2014;18(5):455–60. doi:10.1111/1744-9987.12109

RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v56.3039
Majalah Kedokteran Bandung. 2024;56(1):62–68

Majalah Kedokteran Bandung (MKB)

Received: September 27, 2023
Accepted: June 1, 2023
Available online: March 31, 2024

# Interleukin-6 Level and Neutrophil-Lymphocytes Ratio and Severity of Coronavirus Disease 19

**Armina Latifah, Ida Parwati, Ana Tjandrawati, Nida Suraya**
Department of Clinical Pathology, Faculty of Medicine, Universitas Padjadjaran
Dr. Hasan Sadikin General Hospital Bandung, Indonesia

**Abstract**

Interleukin-6 (IL-6) is a proinflammatory cytokine that plays an important role in hyperinflammation and cytokine storm in Coronavirus Disease 19 (COVID-19) patients. The neutrophil-lymphocyte ratio (NRL) describes the innate and adaptive immune responses. Elevated IL-6 and NLR levels usually indicate a severe clinical condition in COVID-19 patients. Aim of this study was to determine the correlation of IL-6 and NLR with the severity of illness in COVID-19 hospitalized patients. This was a descriptive correlative observational study with a cross-sectional design using secondary data from COVID-19 patients treated in Dr. Hasan Sadikin General Hospital, Bandung, Indonesia, from November 2020 to October 2021. There were 225 subjects who were classified by the severity and analyzed for IL-6 levels and NLR. Median levels of IL-6 at moderate, severe, and critical levels were 4.1 pg/mL, 20.4 pg/mL, and 38.8 pg/mL, respectively. The median NLR at moderate, severe, and critical grades were respectively 4.41, 9.65, and 17.79. The correlation between IL-6, NLR, and severity was 0.441 ($p<0.001$) and 0.408 ($p<0.001$). Meanwhile, the correlation between IL-6 levels and NLR in COVID-19 was 0.230 ($p<0.001$). Thus, IL-6 and NRL levels have a moderate positive correlation with the severity of COVID-19, while IL-6 and NRL have a weak correlation because IL-6 is not the only factor that affects the NRL.

**Keywords:** COVID-19, disease severity, IL-6, NRL

## Introduction

Coronavirus Disease 19 (COVID-19) is a disease caused by the Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). The World Health Organization (WHO) officially declared COVID-19 as a pandemic in March 2020. The number of COVID-19 cases has reached 200 million people with 4 million deaths worldwide in July 2021.[1]

Acute Respiratory Syndrome Coronavirus-2 will infect respiratory epithelial cells through the ACE-2 receptor. Infected epithelial cells induce innate immune cells. Antigen presenting cells (APC) will present viral antigens to T helper cell/ CD4+ lymphocytes, thereby activating these cells. COVID-19 infection will be limited to the lungs and manifests mildly or even without symptoms if the immune response is good, whereas if the immune response is poor, it can cause a cytokine storm, causing severe clinical manifestations that can develop into multiorgan failure (MOF), Acute respiratory distress syndrome (ARDS), and have a poor prognosis. Interleukin-6 is a proinflammatory cytokine that plays a major role in cytokine storm and clinical manifestations of COVID-19 patients.[2-4]

IL-6 examination is very necessary, especially for treatment guidelines, but not all health services have the examination tool. Interleukin-6 released by immune cells can cause dysregulation, fatigue, and apoptosis of lymphocyte cells, resulting in a decrease in lymphocyte cells. Interleukin-6 can increase the production and recruitment of neutrophils and prolong the life span of neutrophils. An increase in neutrophils and a decrease in lymphocytes causes an increase in NLR (Neutrophil lymphocyte ratio). The routine hematological examination is simple and can determine NLR which is one of the markers of inflammation besides IL-6. In

**Corresponding Author:**
Armina Latifah
Department of Clinical Pathology, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital Bandung, Indonesia
Email: armina.latifah@gmail.com

moderate COVID-19 conditions, IL-6 and NLR levels will begin to increase, and IL-6 and NLR levels increase as the disease progresses. This study aims to examine the correlation of IL-6 and NLR with the severity of COVID-19 patients. [5,6]

## Methods

This study used secondary data from subjects with confirmed COVID-19 who were hospitalized at RSUP Dr. Hasan Sadikin Bandung from November 2020 to October 31, 2021. This study received ethical approval from The Research Ethics Committee of Dr. Hasan Sadikin General Hospital Bandung. The data taken are clinical data of research subjects from medical records and laboratory results from the Laboratory Information System (LIS). Hasan Sadikin Bandung. Data on the clinical stage of the patient was taken from the medical record section of Dr. RSUP. Hasan Sadikin Bandung. IL-6 examination and leukocyte count were taken through LIS at the Clinical Pathology section of Dr. RSUP. Hasan Sadikin Bandung. Sampling was based on purposive consecutive sampling. The inclusion criteria for research subjects were patients aged >18 years with confirmed COVID-19 who were examined for IL-6 and leukocyte count on the same day at the beginning of hospital admission with moderate, severe, and critical clinical manifestations. Exclusion criteria in this study were suffering from or having malignancy, autoimmune disease, HIV, aplastic anemia and receiving anti-inflammatory drugs and/or corticosteroids. The number of subjects included in this study was 230 subjects. A total of 3 subjects were excluded because they had a history of malignancy and 2 subjects excluded had a history of autoimmune at the time of recruitment of research subjects. The number of subjects who met the inclusion criteria and did not meet the exclusion criteria was 225 subjects.

The design of this study was a descriptive, correlative cross-sectional study using secondary data. Statistical analysis was carried out to determine the significant correlation between IL-6 levels and NLR values in COVID-19 patients with moderate, severe, and critical stages. The research data were analyzed using the Statistical Package for the Social Science (SPSS) program. The research data were tested for normality using the Kolmogorov Smirnov's test. The levels of IL-6 and NLR in this study had an abnormal distribution, so the Spearman Rank correlation test was carried out. The r coefficient of the Spearman Rank correlation test means that if you get a value of 0.00 – 0.09 then the correlation can be ignored, the value is 0.10 – 0.39 then the correlation is weak, the value is 0.40 – 0.69 then the correlation is moderate, the value is 0.70 –0.89 means a strong correlation, and a value of 0.90–1.00 means a very strong correlation. All of these tests used a 95% confidence level ($\alpha$=0.05) and a p-value of <0.05 was considered statistically significant.

## Results

The results of the normality test for age, leukocyte count, neutrophil count, lymphocyte count, and IL-6 levels were not normally distributed and presented in the form of the median and interquartile range (IQR). The data on the characteristics of the research subjects can be seen in Table 1.

The number of subjects in this study was 225 people, with a median age of 59 years, 63.6% male and 36.4% female. The most severity disease stage was severe (52.0%), and the most comorbid was hypertension (43.6%).

The correlation between IL-6 levels and the degree of severity in research subjects was tested using Spearman's rank test because the data for IL-6 levels were not normally distributed. The results of Spearman's rank test between IL-6 levels and the degree of disease severity in research subjects in tabular form can be seen in Table 2.

The results in Table 2 show that the median IL-6 at moderate was 4.1 pg/mL (IQR: 2.7–10.1 pg/mL), then at severe was 20.4 pg/mL (IQR: 8.6 –49.2 pg/mL) and critical was 38.8 pg/mL (IQR: 15.3–123.8 pg/mL).

The correlation between NLR levels and severity in research subjects was tested using Spearman's rank test because the data for NLR levels were not normally distributed. The results of Spearman's rank test between NLR and the degree of disease severity in research subjects can be seen in Table 3.

The results in Table 3 show that the median NLR at moderate was 4.41 (IQR: 3.59–11.17), severe was 9.65 (IQR: 5.39–18.39), and critical was 9.65. 17.79 (IQR: 9.47–25.27). The boxplot graph of the correlation between IL-6 and NLR with severity can be seen in Figure 1 below

In the bloxspot image above, there is a positive correlation with moderate strength between IL-6 and severity (r=0.441, p<0.001), and there is a positive correlation with moderates strength

**Table 1 Subject Characteristics**

| Variable | Amount (n=225) |
|---|---|
| Age (years), median (IQR) | 59 (49-67) |
| Gender, n (%) | |
| Man | 143 (63.6) |
| Woman | 82 (36.4) |
| Severity, n (%) | |
| Moderate | 38 (16.9) |
| Severe | 117 (52.0) |
| Critical | 70 (31.1) |
| Comorbid disease, n (%) | |
| Diabetes mellitus | 66 (29.3) |
| Hypertension | 98 (43.6) |
| Obesity | 19 (8.4) |
| Heart disease | 63 (28.0) |
| Leukocyte Count/μL, median (IQR) | 10.780 (6.850–15.675) |
| Neutrophil Count/μL, median (IQR) | 8.600 (5.415–14.480) |
| Lymphocyte CountL, median (IQR) | 820 (545–1.300) |
| NLR, median (IQR) | 10.61 (5.26–20.21) |
| IL-6 (pg/mL), median (IQR) | 20.40 (6.25–55.75) |

Note: n=frequency, %=percentage, SD=*Standard Deviation*, IQR=*Interquartile Range*

**Table 2 Correlation of IL-6 Levels with the Severity of COVID-19**

| Variable | Severity | | | r coefficient | p-value |
|---|---|---|---|---|---|
| | Moderate | Severe | Critical | | |
| IL-6 Median (IQR) pg/mL | 4.1 (2.7 – 10.1) | 20.4 (8.6 – 49.2) | 38.8 (15.3 – 123.8) | 0.441 | <0.001* |

Note: IQR=Interquartile Range data analysis using Spearman's rank correlation test, r = Spearman correlation coefficient
*p<0.05=significant

**Table 3 Correlation of NLR Levels with the Severity of COVID-19**

| Variable | Severity | | | | p value |
|---|---|---|---|---|---|
| | Moderate | Severe | Critical | r coefficient | |
| NLR Median (IQR) | 4.41 (3.59 – 11.17) | 9.65 (5.39 – 18.39) | 17.79 (9.47 – 25.27) | 0.408 | <0.001* |

Note: IQR=Interquartile Range data analysis using Spearman's rank correlation test, r = Spearman correlation coefficient
*p<0.05=significant

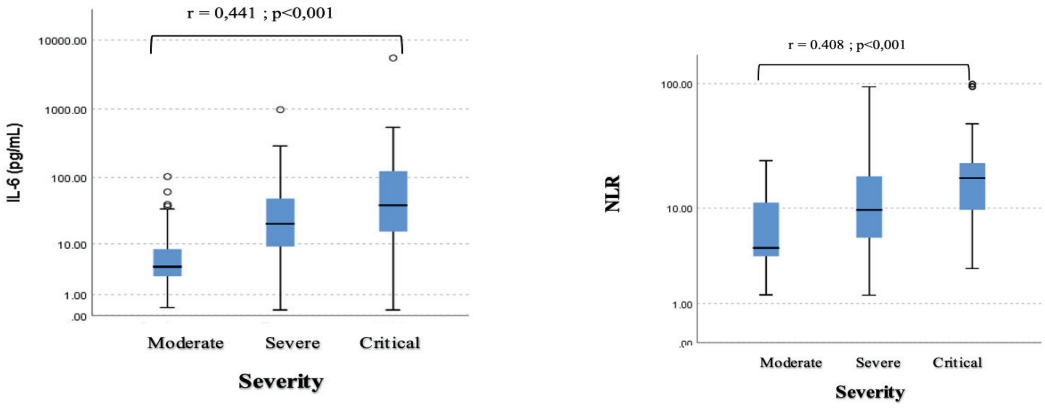


**Figure 1 Boxplot Distribution of IL-6 and NLR by Severity of Disease**

between NLR and severity in study subjects (r=0.408, p <0.001). The increase in IL-6 levels or NLR values, the more severe the severity of COVID-19.

The correlation between IL-6 levels and NLR in research subjects was tested by Spearman's rank test because IL-6 and NLR data not normally distributed. The results of the analysis using Spearman's rank correlation showed that there was a positive correlation with weak strength between IL-6 and NLR (r=0.230, p<0.001).

## Discussion

Median age in this study was 59 years. Research Silva et al. in Brazil patients treated with moderate to severe degrees had a median age of 58.3 years. In this study, 166 subjects (71%) were older than 50 years. People over 50 years old tend to experience changes in function and cellular composition of innate and adaptive immunity (immunosenescence). Changes in T and B cell function and an increase in cytokine production causing people in that age tend to have more severe symptoms that they need hospitalized. At that age there is also an increased risk of developing comorbid diseases such as hypertension, diabetes mellitus, and cardiovascular disease.[7,8]

Researchers took the four most common comorbid diseases causing the risk of complications and death in COVID-19 based on previous research, namely hypertension, diabetes mellitus, cardiovascular and obesity. This study's most comorbid subjects were hypertension (43.6%). According to the study of Richardson et al.[9] in America and Zhou F et al.[10] in China, patients with hypertension were the most comorbid in COVID-19 patients. In hypertensive patients, an imbalance in the renin-angiotensin system can cause inflammation, thrombus, and other side effects that aggravate COVID-19 patients so that COVID-19 patients with hypertension tend to be treated. In addition, chronic hypertension can lead to decreased cardiovascular reserve, arterial wall stiffness, endothelial dysfunction, and left ventricular hypertrophy, making these patients susceptible to complications from COVID-19, such as myocardial injury, thrombosis, and acute kidney injury (AKI).[9,10]

The results of the examination of IL-6 levels in all study subjects had a median of 20.40 pg/mL. The researcher divided the subjects into three categories based on the severity of COVID-19 disease: moderate, severe, and critical. Subjects with a median grade of 4.1 pg/mL for IL-6 levels, subjects with severe degrees of IL-6 levels with a median of 20.4 pg/mL, and subjects with a critical grade with a median of 38.8 pg/mL. The results of the correlation analysis between IL-6 levels and severity showed a moderate positive correlation statistically (r=0.441, p<0.001). The correlation indicates that the more severe the severity, the higher the IL-6. The positive correlation of IL-6 with the severity of COVID-19 patients was similar to the meta-analysis study of Liu et al.[11] 2021 and Andriani et al.[12] in Indonesia. In the research of Liu et al., They found that the Spearman correlation has a value of r = 0.709 with p = 0.002 which means it has a strong correlation, while in this study, there is a moderate correlation. This study is because

there are differences in the research of Liu et al.[11] The characteristics of the study subjects were more varied than this study, only dividing the two groups between severe and not severe and not excluding the use of anti-inflammatory drugs and corticosteroids. In the research of Andriani et al[12], the Spearman correlation value r = 0.337 with p = 0.017 means it has a weak correlation compared to this study. The difference in this study is the number of subjects. The study from Andriani et al.[12] had 50 subjects, including subjects with mild degrees, in the analysis, and the use of anti-inflammatory and corticosteroids was not excluded. The progression of COVID-19 disease from severe to critical is thought to be strongly associated with a cytokine storm. Cytokine storms not only cause damage to epithelial and endothelial cells but also cause vascular leakage, which ultimately leads to ARDS and other severe conditions. In critically ill COVID-19 patients, there is abnormal production and regulation of proinflammatory cytokines. Proinflammatory cytokines that play a role in cytokine storm is IL-6. Interleukin-6 can induce other proinflammatory cytokines in immune cells, recruiting leukocytes and increasing migration from blood to organs, thereby aggravating the inflammatory state. Although there was a moderate correlation in this study, IL-6 is a proinflammatory cytokine that plays a crucial role during a cytokine storm. Other factors can influence disease development, such as other inflammatory cytokines (TNFα, IFN type 1, and IL-10), the condition of immune cells, and comorbidities in patients.[11-15]

The results of the correlation analysis between NLR and severity showed a moderate positive correlation which was statistically significant (r=0.408, p<0.001). The correlation shows that the more severe the severity, the higher the NLR value. This study followed the research of Suartono et al.[16] 2021 in Indonesia (r=564, p<0.001), which states that the NLR value has a moderate correlation to the severity of COVID-19 patients. The higher neutrophil-lymphocyte ratio is due to an increase in the number of neutrophils and a decrease in lymphocytes. The neutrophil-lymphocyte ratio reflects the balance between innate immunity (neutrophils) and adaptive immunity (lymphocytes). A higher NLR indicates that in severe cases, a dysregulated immune response indicates an overactive innate immune tendency in the immune system. This exaggerated inflammatory response can exacerbate the cytokine storm and increase tissue damage. Although neutrophils are part of innate immunity and lymphocytes are part of the adaptive immune system, many other components of the immune system influence the state of infection and the severity of COVID-19 patients. The components of innate immunity consist of epithelial cells, dendritic cells, natural killer cells, phagocytes, macrophages, and complement proteins. The main components of the adaptive immune system consist of lymphocytes and antibodies.[16-18]

The results of the correlation analysis between IL-6 and NLR levels showed a weak positive correlation which was statistically significant (r=0.230, p<0.001). In this study, there is a positive correlation following the research of Garcia-Gordillo et al.[19] in Mexico, Sayah et al.[20] in Algeria, and Liu et al.[17] in China. In the study of Garcia-Gordillo et al.[19] in Mexico (r = 0.485, p < 0.001) and Sayah et al.[20] in Algeria (r=0.634, p<0.001) which had a moderate correlation between IL-6 and NLR while this study it had a weak correlation, this may be due to differences in the inclusion and exclusion criteria. According to research by Liu et al.[17] in China (r=0.359, p<0.005), IL-6 had a weak correlation with NLR. Interleukin-6 is known to increase the production of maturation and activation of neutrophils and stimulate lymphocyte apoptosis to increase the value of NLR. Neutrophils are also one of the producers of IL-6, although not the main one. The correlation between IL-6 and NLR is weak because other processes influence the increase in neutrophils and the decrease in lymphocytes besides the increase in IL-6. The production and activation of neutrophils increased, also influenced by GM-CSF, IL-17, and Th17 cells and the presence of secondary infection. The reduction of lymphocytes caused by fatigue and lymphocyte apoptosis is not only affected by IL-6; other cytokines, such as IL-10 and TNF- can stimulate lymphocyte fatigue and apoptosis. SARS-CoV-2 that binds directly to the ACE-2 receptor on lymphocytes may also interfere with lymphocyte function directly. Lung damage caused by COVID-19 causes the accumulation of lactic acid in the body, which can interfere with lymphocyte function and cause a decrease in lymphocytes.[5,17,19,20]

The limitation of the study is that the researcher did not know the history of vaccination and the SARS-CoV-2 variant. The researcher took the study at one time without seeing any changes in the severity status of the study subjects during treatment.

This study concludes that IL-6 levels and NLR values had a moderately positive correlation with the severity of COVID-19 while the correlation

between IL-6 and NLR in COVID-19 patients is weak because IL-6 is not the only factor affecting NLR. Interleukin-6 and NLR examinations are expected to increase clinician awareness in managing COVID-19.

## References


1. IRIS. COVID-19 weekly epidemiological update. 50 ed. Geneva: World Health Organization; 2021.
2. Bohn MK, Hall A, Sepiashvili L, Jung B, Steele S, Adeli K. Pathophysiology of COVID-19: mechanisms underlying disease severity and progression. Physiology (Bethesda). 2020;35(5):288–301. doi:10.1152/physiol.00019.2020
3. Burhan E, Susanto AD, Isbaniah F, Nasution SA, Ginanjar E, Pitoyo CW, et al, Editors. Pedoman Tatalaksana COVID-19. 4 ed. Jakarta: PDPI, PERKI, PAPDI, PERDATIN, IDAI; 2022.
4. Murphy K, Weaver C, Editors. Janeway's immunobiology. 9th ed. New york: Garland science; 2016.
5. Yang L, Liu S, Liu J, Zhang Z, Wan X, Huang B, et al. COVID-19: immunopathogenesis and Immunotherapeutics. Signal transduction and targeted therapy. 2020;5(1):1–8.
6. Yang P-H, Ding Y-B, Xu Z, Pu R, Li P, Yan J, et al. Increased circulating level of interleukin-6 and CD8+ T cell exhaustion are associated with progression of COVID-19. Infectious Diseases of Poverty. 2020;9(1):161. doi:10.1186/s40249-020-00780-6
7. Silva B, Rodrigues W, Abadia D, Alves da Silva D, Andrade E. Clinical-Epidemiology Aspect of Inpatients With Moderate or Severe COVID-19 in a Brazilian Macroregion: Disease and Countermeasures. Front Cell Infect Microbiol. 2022;12:899702. doi:10.3389/fcimb.2022.899702
8. Jameson JL, Kasper DL, Longo DL, Fauci AS, Hauser SL, Loscalzo J, Editors. Harrison's principles of internal medicine. 20th ed. United States: McGraw-Hill; 2018.
9. Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City Area. JAMA. 2020;323(20):2052–9. doi:10.1001/jama.2020.6775
10. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395(10229):1054–62. doi:10.1016/s0140-6736(20)30566-3
11. Liu X, Wang H, Shi S, Xiao J. Association between IL-6 and severe disease and mortality in COVID-19 disease: a systematic review and meta-analysis. Postgraduate Medical Journal. 2021:postgradmedj-2021-139939. doi:10.1136/postgradmedj-2021-139939
12. Andriani I, Utariani A, Hanindito E, Airlangga PS. Correlation between CRP Il-6 level, severity and mortality in patients with Covid-19 infection in Indonesia. International J Scientific Advances. 2021;2(4):2708.
13. Hashizume M. Outlook of IL-6 signaling blockade for COVID-19 pneumonia. Inflammation and Regeneration. 2020;40(1):24. doi:10.1186/s41232-020-00134-7
14. Skevaki C, Fragkou PC, Cheng C, Xie M, Renz H. Laboratory characteristics of patients infected with the novel SARS-CoV-2 virus. J Infection. 2020;81(2):205–12. doi:https://doi.org/10.1016/j.jinf.2020.06.039
15. Henry BM, Oliveira MHSd, Benoit S, Plebani M, Lippi G. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. Clin Chem Lab Med. 2020;58(7):1021–28. doi:doi:10.1515/cclm-2020-0369
16. Suhartono S, Wijaya I, Dalimoenthe NZ. The correlation of neutrophil-to-lymphocyte ratio (NLR) and monocytes-to-lymphocytes ratio (MLR) with disease severity in hospitalized patients with Coronavirus disease 2019 (COVID-19). Bali Med J. 2021;10(2):653–8.
17. Liu L, Zheng Y, Cai L, Wu W, Tang S, Ding Y, et al. Neutrophil-to-lymphocyte ratio, a critical predictor for assessment of disease severity in patients with COVID-19. International journal of laboratory hematology. 2021;43(2):329–35.
18. Lanier L. The innate and adaptive immune systems. In: Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P, Editors. Molecular biology of the cell. 6 ed. New York: Garland Science; 2015. p. 1297–39.
19. Garcia-Gordillo JA, Camiro-Zúñiga A, Aguilar-Soto M, Cuenca D, Cadena-Fernández A, Khouri LS, et al. COVID-IRS: A novel predictive score for risk of invasive mechanical ventilation in patients with COVID-19. PLOS

ONE. 2021;16(4):e0248357. doi:10.1371/journal.pone.0248357

20. Sayah W, Berkane I, Guermache I, Sabri M, Lakhal FZ, Yasmine Rahali S, et al. Interleukin-6, procalcitonin and neutrophil-to-lymphocyte ratio: Potential immune-inflammatory parameters to identify severe and fatal forms of COVID-19. Cytokine. 2021;141:155428. doi:10.1016/j.cyto.2021.155428

CASE REPORT

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v56.3334
Majalah Kedokteran Bandung. 2024;56(1):69–72

Majalah Kedokteran Bandung (MKB)

Received: May 6, 2023
Accepted: November 21, 2023
Available online: March 31, 2024

# Bilateral Optic Nerve Atrophy Case Report

**Mohammed R. Naqeeb, Dina M. Abdulmannan**
College of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia

**Abstract**

Wolfram syndrome, also known as DIDMOAD or juvenile onset diabetes mellitus, optic nerve atrophy, diabetes insipidus, and deafness, is a genetic neurological condition. This case report provides a description on the first instance of Wolfram syndrome in a Saudi family, which manifested as proliferative diabetic retinopathy and a powder-like cataract, among the other unusual ophthalmological findings. This case involved a 27-year-old Saudi woman with bilateral optic nerve atrophy who was first diagnosed with diabetes mellitus at the age of 8 years. At the age of 18, bilateral optic nerve atrophy was identified. At the age of 27, diabetes insipidus and hearing loss were verified. There were no signs of renal, neurological, or psychiatric issues. Atypical ophthalmological traits were examined and addressed in this study. Any individual with bilateral optic nerve atrophy and insulin-dependent diabetes mellitus within the first 30 years of life should be evaluated for the possibility of Wolfram syndrome. Microvascular diabetes is an incredibly rare complication of Wolfram syndrome. Early diagnosis, treatment, and prevention of severe consequences can result in improved survival rates and quality of life.

**Keywords:** Atrophy, optic nerve, wolfram syndrome, Saudi Arabia

## Introduction

Wolfram made the first observation in 1938, and Wagener later explained it. A complicated, uncommon, autosomal recessive neurological illness, Wolfram syndrome (MIM222300) is inherited. It is also known as DIDMOAD (diabetes insipidus, diabetes mellitus, optic atrophy, and deafness) and is defined by juvenile onset non-immune insulin dependent diabetes mellitus, progressive bilateral optic nerve atrophy, and sensorineural hearing loss.[1,2,3] Urinary tract, neurological, reproductive, and psychological problems may be linked to Wolfram syndrome. This disease's pathophysiology is still a mystery.[1,2] The wolfram syndrome mutant gene (WFS1) has been located on chromosome 4p 16.1, however there is evidence of locus heterogeneity and mutations in mitochondrial genes in a small number of documented cases.[4,5] In the UK, 1/770.000 was reported as the prevalence rate.[6] According to several publications, the proportion of individuals with a full Wolfram syndrome varies between 13% and 53%. Less than 8% of the 300 patients described in the literature from all around the globe had diabetic retinopathy as a side effect of wolfram syndrome.[1] There are, however, just a few sources that describe the relationship of cataracts with this disease. This case report describes the first case of Wolfram syndrome in a Saudi family, which manifested as proliferative diabetic retinopathy and a powder-like cataract, among other uncommon ophthalmological findings. The aim of this case report was to report the case of Saudi female patient who was diagnosed with diabetes mellitus, bilateral optic nerve atrophy, and hearing loss.

## Case

A 27-year-old Saudi woman with bilateral optic nerve atrophy was sent to the eye clinic at King Abdullah Medical City. Her medical history showed that both of her eyes' visual acuity had gradually declined beginning at age seven, and at age eighteen, she had been identified as having bilateral optic nerve atrophy. She was first diagnosed with insulin-dependent diabetes mellitus when she was eight years old. Despite using insulin, she frequently had uncontrolled

**Corresponding Author:**
Dr. Mohammed R. Naqeeb
College of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia
Email: mrnaqeeb@uqu.edu.sa

blood sugar. At the age of 20, she disclosed a history of polyuria and polydipsia, but she didn't seek medical attention. Her consanguineous parents' good family history was crucial. Her older sister, 35, has had insulin-dependent diabetes since she was 10 years old. Later, she experienced hearing and vision problems. Her younger sister and brother, however, were in good health. Her greatest corrected visual acuity was found to be counting fingers in both eyes during assessment. At the age of twenty-seven, she was legally blind and had the maximum corrected visual acuity of counting digits at a distance of two meters. Clinical and biochemical tests, including urine and serum osmolality measurements and the water-deprivation test, all supported the diagnosis of diabetes insipidus of central origin. Her eldest sister had a significant familial history of hearing loss. Our audiogram, which was typical for hearing loss at medium to high frequencies, revealed sensorineural hearing loss. The intraocular pressure in the right eye was 14 mmHg, whereas the intraocular pressure in the left eye was 16 mmHg. RAPD was not found in either of the eyes. Slit lamp examination revealed cataracts that resembled powder during lamellar tiny spots opacity in both lenses. A fundus examination revealed a bilateral pale disc that was a sign of optic atrophy. Additionally, the macula in both eyes displayed some hard exudate, a microaneurysm, and hemorrhage as a result of diabetic retinopathy. Only the right eye had proliferative diabetic retinopathy, which caused preretinal hemorrhage and other neovascularization. The patient received a thorough radiological and laboratory assessment, which included normal US scans of the kidney and bladder as well as the kidney and orbit on CT and MRI. According to osmometer measurements, urine's osmolality was low (170 mosm/kg) and serum's was high (305 mosm/kg). The results of the water-deprivation test supported central diabetic insipidus. Analyses of the urine revealed microalbuminuria. An audiogram with moderate to severe sloping and symmetric sensorineural hearing loss in both ears, with a preference for medium to high frequencies, was found. In this case, no signs of renal, neurological, reproductive, or psychological problems were found. Regarding her older sister, a fundus exam revealed bilateral pale optic discs indicative of optic atrophy and no changes associated with diabetic retinopathy. She wasn't accessible for a second assessment, despite our suspicions that she could have Wolfram syndrome.

This study used de-identified data and was considered exempt from human protection oversight by the institutional review board. Informed consent to participate was obtained from the patient.

## Discussion

In the early phases of the illness, wolfram syndrome diagnosis is not usually straightforward. The possibility of juvenile onset diabetes mellitus and an atrophying optic nerve is the suspicion. According to previous literature [2], these two characteristics are rather stable (99% for DM and 97.5% for optic atrophy), in contrast to the other signs and symptoms, which are highly varied in terms of age of start and severity. Before the age of ten, non-immune type 1 diabetes usually manifests as Wolfram syndrome. Diabetes mellitus linked with wolfram syndrome has the same clinical characteristics as type 1 diabetes mellitus. However, the main distinction between autoimmune diabetes and typical type 1 diabetes mellitus is that the former lacks a particular immune or genetic component. The fact that glycemic control is simpler to achieve in wolfram syndrome is another significant distinction from type 1 diabetes mellitus as a whole [1]. Except in extremely rare circumstances, wolfram syndrome does not typically exhibit any symptoms of diabetic retinopathy.[7,8] Diabetic retinopathy was uncommon and, when it was present, proceeded more slowly than anticipated in patients with long-term diabetes mellitus (>15 years). The frequency of diabetic retinopathy in wolfram syndrome was 8%.[7,8] The more manageable nature of diabetes mellitus in wolfram syndrome may have contributed to the previous study's finding that diabetic retinopathy is on the decline [7,8]. There have been reports of a few more severe proliferative retinopathies. Here, we present the case of a patient with wolfram syndrome who was diagnosed 27 years after developing insulin-dependent diabetes at the age of 8. At the age of 27, she experienced preretinal hemorrhage in her right eye as well as proliferative diabetic retinopathy in both of her eyes. She had laser therapy (also known as pan retinal photocoagulation). Optic nerve atrophy, a defining feature of Wolfram syndrome, often began before the age of 15. The average age of the patients was 13.1, with a range of 6 to 30 years.[9-12] Optic atrophy was the first presenting symptom in just 9% of wolfram syndrome patients [1,8]. Optic atrophy often results in a fairly severe vision

impairment, with young adults having visual acuities between 20/200 and 20/400. Total blindness in wolfram syndrome is uncommon, and it takes seven years for considerable vision loss to be considered legally blind. Severe axon loss and demyelination of the optic nerve, chiasm, and tracks are the main causes of blindness. The vision issues in our situation began at a young age of seven. At the age of 18, the patient was identified as having bilateral optic nerve atrophy. She was legally blind at the age of twenty-seven and had the greatest corrected visual acuity of counting fingers at a distance of two meters. Rarely is the occurrence of lens opacity linked to wolfram syndrome mentioned in literature. There are succinct and cryptic statements that fail to identify a specific kind of lens opacity [1]. At the age of 27, we discovered lens opacity in both of our patient's eyes, which was characterized as looking like powder. One instance with the same kind of lens opacity has been documented in the literature.[1,8] Wolfram syndrome does not always include diabetes insipidus, and symptoms typically appear in the second or third decade (between 3 and 40 years old) and are often brought on by central origin abnormalities rather than nephrogenic causes. The atrophy and gliosis of the whole supraoptic and paraventricular neurohypophysaroious system, which results in a deficit in vasopressin, which is responsible for the concentration of urine, was shown by histological and clinical investigations to be the cause of this problem.[10,11,13] This patient's modest polyuria and polydipsia, two signs of diabetes insipidus, first appeared when she was twenty years old. At age 27, she began to experience mild symptoms like polyuria, polydipsia, and nocturia. Clinical and biochemical tests, such as urine and serum osmolality measurements as well as the water-deprivation test, all supported the diagnosis of central origin diabetes insipidus. Due to the fact that it has no impact on voice frequencies, sensorineural hearing loss is often not apparent in adolescents and young adults. When an audiogram revealed severe high frequency loss without any symptoms, as we saw in our instance, it might be a useful diagnostic tool. These individuals' deafness is neurological in nature, affecting the auditory nerve and its central route. Degenerative atrophy of the vestibulocochlear nuclei and inferior colliculi causes a reduction in auditory perception rather than a problem with sound transmission to the nerve.[5,11] The majority of the cases have high frequency hearing loss, which, while it does not affect the ability to hear voices, may have important implications for career counseling.[5]

In this instance, the patient denied having any hearing issues. However, as was mentioned above, she had a strong family history of hearing loss in her older sister. The audiogram in our situation, which was typical for medium to high frequency hearing loss, revealed sensorineural hearing loss. Urinary tract abnormalities, hypogonadism, complications of the central nervous system such ataxia and nystagmus, and mental disorders are prone to develop as people age and should be tested for on a regular basis. In these patients, the median death age is 28 years, and up to 60% of cases pass away by the time they are 35 years old.[9,10] Following brain stem atrophy, central respiratory failure is the primary cause of death. Unfortunately, there is currently no cure for Wolfram syndrome patients to stop the underlying neurodegenerative process. The significant differential diagnoses for wolfram syndrome that need to be ruled out are Friedreich's ataxia, Leber's hereditary optic atrophy, and Behr's optic atrophy.

Bilateral optic nerve atrophy is a pathological disorder characterized by the progressive degeneration of the optic nerves, resulting in the impairment or loss of visual function. The process of establishing a definitive diagnosis encompasses a comprehensive approach that includes clinical evaluation, assessment of medical history, and utilization of several diagnostic techniques such as Visual Field Testing, Visual Acuity Testing, Ophthalmoscopy/ Funduscopy, Optical Coherence Tomography, and Magnetic Resonance Imaging.

In conclusion, anyone who has bilateral optic nerve atrophy and insulin-dependent diabetic mellitus during the first three decades of life should be evaluated for the possibility of Wolfram syndrome. An extremely uncommon consequence of wolfram syndrome is micro vascular diabetes. Improved survival rates and quality of life may result from early diagnosis, treatment, and prevention of major consequences. The comprehensive documentation of the patient's diagnostic process, encompassing the encountered problems in determining the etiology of optic nerve atrophy and hearing loss, can aid healthcare professionals in enhancing their diagnostic methodologies and exploring a wider spectrum of potential causative elements.

## References

1. Maleki N, Bashardoust B, Zakeri A, Salehifar

A, Tavosi Z. Diabetes mellitus, diabetes insipidus, optic atrophy, and deafness: A case of Wolfram (DIDMOAD) syndrome. J Curr Ophthalmol. 2016;27:132–5.
2. Pallotta MT, Tascini, G., Crispoldi, R, Orabona C, Mondanelli G, Grohmann U, Esposito, S. Wolfram syndrome, a rare neurodegenerative disease: from pathogenesis to future treatment perspectives. Journal of translational medicine. 2019;17(1):1–12.
3. Urano F. Wolfram Syndrome: Diagnosis, management, and treatment. Current Diabetes Reports. 2016;16(1):6. doi:10.1007/s11892-015-0702-6
4. Delvecchio M, Ortolani F, Palumbo O, Aloi C, Salina A, Susca FC, et al. A Novel Genetic Variant in the WFS1 Gene in a Patient with Partial Uniparental Mero-Isodisomy of Chromosome 4. Int J Mol Sci. 2021;22(15):8082. doi:10.3390/ijms22158082
5. Shi SM, Han YH, Wang HB. Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi. 2016;51(9):712–5. doi:10.3760/cma.j.issn.1673-0860.2016.09.019
6. Zmysłowska A, Borowiec M, Fendler W, Jarosz-Chobot P, Myśliwiec M, Szadkowska A, et al. The prevalence of Wolfram syndrome in a paediatric population with diabetes. Endokrynol Pol. 2014;65(4):295–7. doi:10.5603/EP.2014.0040
7. Barboni P, Amore G, Cascavilla ML, Battista M, Frontino G, Romagnoli M, et al. The pattern of retinal ganglion cell loss in wolfram syndrome is distinct from mitochondrial optic neuropathies. Am J Ophthalmol. 2022;241:206–16. doi:10.1016/j.ajo.2022.03.019
8. Soares A, Mota Á, Fonseca S, Faria O, Brandão E, Falcão Dos Reis F, et al. Ophthalmologic Manifestations of Wolfram Syndrome: Report of 14 Cases. Ophthalmologica. 2019;241(2):116–9. doi:10.1159/000490535
9. Iafusco D, Zanfardino A, Piscopo A, Curto S, Troncone A, Chianese A, et al. Metabolic treatment of wolfram syndrome. Int J Environ Res Public Health. 2022;19(5):2755. doi:10.3390/ijerph19052755
10. Salzano G, Rigoli L, Valenzise M, Chimenz R, Passanisi S, Lombardo F. Clinical Peculiarities in a Cohort of Patients with Wolfram Syndrome 1. Int J Environ Res Public Health. 2022;19(1):520. doi:10.3390/ijerph19010520
11. Samara A, Lugar HM, Hershey T, Shimony JS. Longitudinal Assessment of Neuroradiologic Features in Wolfram Syndrome. AJNR Am J Neuroradiol. 2020;41(12):2364–9. doi:10.3174/ajnr.A6831
12. Tarała W, Drachal E, Mazur A, Korczowski B, Szadkowska A, Zmysłowska A, et al. Wolfram Syndrome. Case report. Przypadek zespołu Wolframa. Pediatr Endocrinol Diabetes Metab. 2016;22(1):39–42. doi:10.18544/PEDM-22.01.0049
13. Kurimoto J, Takagi H, Miyata T, Hodai Y, Kawaguchi Y, Hagiwara D, et al. Deficiency of WFS1 leads to the impairment of AVP secretion under dehydration in male mice. Pituitary. 2021;24(4):582–8. doi:10.1007/s11102-021-01135-6