E-ISSN: 2716-0920
P-ISSN: 0215-8833

MAJALAH BIOMORFOLOGI

Biomorphology Journal

Vol. 34 No. 2 July 2024

Published by: Universitas Airlangga
Jl. Dr. Ir. H. Soekarno, Mulyorejo, Kec. Mulyorejo,
Surabaya, Indonesia 60115
in collaboration with
(Ikatan Dokter Indonesia - IDI Surabaya)
(PAAI- Perhimpunan Ahli Anatomi Indonesia)

Vol. 34 No. 2 July 2024

pISSN 0215-8833
eISSN 2716-0920

MAJALAH BIOMORFOLOGI (BIOMORPHOLOGY JOURNAL)

EDITORIAL TEAM

Address

Faculty of Medicine, Universitas Airlangga
Jl. Mayjen Prof Dr Moestopo no. 47, Surabaya 60131, Indonesia. Phone: 62-31-5020569 ext. 1199
https://e-journal.unair.ac.id/MBIO/
Email: mbiomj@fk.unair.ac.id

Vol. 34 No. 2 July 2024

pISSN 0215-8833
eISSN 2716-0920

MAJALAH BIOMORFOLOGI (BIOMORPHOLOGY JOURNAL)

CONTENT

ORIGINAL RESEARCH REPORT

SYSTEMATIC REVIEW

SCOPING REVIEW

ORIGINAL RESEARCH REPORT

Depletion of Lysyl Oxidase-Like 4 (LOXL4) Attenuates Colony Formation *in vitro* and Collagen Deposition *in vivo* Breast Cancer Model

Ni Luh Gede Yoni Komalasari[1,2*], I Gde Haryo Ganesha[1], I Gusti Nyoman Sri Wiryawan[1], Nahoko Tomonobu[2], Masakiyo Sakaguchi[2]

[1]Department of Histology, Faculty of Medicine, Udayana University, Bali, Indonesia.
[2]Department of Cell Biology, Graduate School of Medicine, Dentistry, and Pharmaceutical Science, Okayama University, Okayama, Japan.

Article Info

Article history:
Received 29-01-2024
Revised 17-04-2024
Accepted 08-05-2024
Published 01-07-2024

Keywords:
Good health
Lysyl oxidase
Extracellular matrix

*__Corresponding author__:
Ni Luh Gede Yoni Komalasari
yonikomalasari@unud.ac.id

ABSTRACT

Background: Lysyl oxidase (LOX) family proteins have recently become a topic in cancer progression. Our recent study found a high LOX-like 4 (LOXL4) expression in MDA-MB-231 cells. **Objective**: To reveal the impact of depleted LOXL4 in both *in vitro* and *in vivo* breast cancer models from a histological perspective. **Material and Method**: Endogenous LOXL4 was depleted using the CRISPR/Cas9 on MDA-MB-231 parental cells. Based on the LOXL4 protein expression, the clone was determined for the next experiment, thus generating MDA-MB-231 LOXL4 KO. Cell assay was conducted using colony formation assay (n=3) followed by crystal violet staining. The indicated cells were inoculated orthotopically to female BALB/c nude mice (n=5). At the end of the experiment, tumors were isolated, fixed, and prepared for Masson's Trichrome staining. **Result**: CRISPR/Cas9 completely depleted LOXL4 expression on clone number #2-22. Depletion of LOXL4 reduced the colony size formed by MDA-MB-231 cells. MDA-MB-231 LOXL4 KO #2-22 derived tumors showed depressed tumor volume compared to the parental group. Reduced collagen was also observed from the Masson's Trichrome staining ($p<0.001$). **Conclusion:** Depletion of LOXL4 downregulates the growth of MDA-MB-231 cells *in vitro* and collagen deposition *in vivo*.

How to cite:
Komalasari, N.L.G.Y., Ganesha, I.G.H, Wiryawan, I.G.N.S, et al. 2024. Depletion of Lysyl Oxidase-Like 4 (LOXL4) Attenuates Colony Formation *in vitro* and Collagen Deposition *in vivo* Breast Cancer Model. Majalah Biomorfologi-Biomorphology Journal, 34(2): 67-73.

Majalah Biomorfologi (Biomorphology Journal) p.ISSN:0215-8833, e.ISSN: 2716-0920
doi: 10.20473/mbiom.v34i2.2024.67-73

Highlights

1. Revealing LOX family members' mechanisms in promoting invasive breast cancer progression is essential for targeting specific molecules in invasive breast cancer.
2. Depletion of LOXL4 in invasive breast cancer shows attenuation of cell invasiveness *in vitro* and collagen deposition in tumor models *in vivo*.

BACKGROUND

Breast cancer is still the most prevalent cancer, with increasing rates along with age. According to the World Health Organization (WHO) data, 2.3 million women were diagnosed with breast cancer, resulting in 685.000 deaths (World Health Organization, 2024). In Asia, breast cancer is the most common cancer in women across most of the countries with high mortality-to-incidence (M/I) of 0.28 compared to the other parts of the world (Huang, et al., 2022; Lim, et al., 2022; Rajappa, et al., 2023). Breast cancer is still the leading cause of mortality related to cancer in the Southeast Asia region and the highest disability-adjusted life years (DALYs) among females (10.9 million). Cancer incidence increases along with life expectancy (Sharma, et al., 2024). Therefore, early detection and target therapy are still essential to be investigated to ease the burden of breast cancer.

In invasive cancer that develops from a solid tumor, breast cancer relies on microenvironment changes, including the extracellular matrix, blood vessels, and immune system (Brassart-Pasco, et al., 2020; Winkler, et al., 2020). Thus, searching for genes related to the extracellular matrix (ECM) for target therapy in breast cancer, we observed intriguing genes as members of the lysyl oxidase (LOX) family (Komalasari, et al., 2023). The lysyl oxidase (LOX) family comprises five members, including LOX, LOX-Like 1 (LOXL1), LOXL2, LOXL3, and LOXL4, which, in general, has been known to contribute to the maturation of collagen and elastin in the ECM (Grau-Bové, et al., 2015; Vallet & Ricard-Blum, 2019). To conduct the function, they depend on the presence of copper (Oldfield, et al., 2018). In recent studies, some LOX family members were observed also to have functions in cancer progression other than the ECM function. LOX is related to hypoxia and tumor invasion in bone (Reynaud, et al., 2017). LOXL2 was observed to induce tumor progression in esophageal squamous cell carcinoma (ESCC) and renal cell carcinoma (RCC) (Hong &Yu, 2019; Liu, et al., 2023). LOXL3 and LOXL4 were reported to promote the growth and invasiveness of melanoma, gastric cancer, and hepatocellular carcinoma (HCC) (Li, et al., 2019; Koorman, et al., 2022). Many studies have presented LOX family members in various cancers, but the mechanisms are still puzzling (Choi, et al., 2017; Shao, et al., 2019). There are some extracellular and intracellular mechanisms in which LOX family members promote tumor progression.

In our previous investigation, high expression of LOX-Like 4 (LOXL4) was found particularly in triple-negative breast cancer (TNBC) cells (Komalasari, et al., 2023). Additionally, low levels of LOXL1 and LOXL4 were observed in less aggressive MDA-MB-231 that were afflicted by ZEB1 mutation (Hirabayashi, et al., 2023). Some studies highlighted the importance of the LOX family members in various cancers. The inhibitions of LOX family members stalled the cancer progression (Chen, et al., 2020). However, which LOX family members are important, and the molecular mechanisms, especially in breast cancer progression, remain to be elucidated. Discovery of the specific molecule and the mechanisms related to breast cancer progression leads to a potential for target therapy, particularly for invasive breast cancer.

OBJECTIVE

In this study, we were interested in distinguishing the LOXL4 function through the depletion of LOXL4 in the TNBC cell line. First, to observe the impact of LOXL4 depletion on the cell function *in vitro*, and second, to use animal models to observe the effect of LOXL4 depletion from a histological perspective. It is essential to identify specific LOX family members that support solid tumor progression by establishing potential beneficial treatments to maintain the well-being of cancer patients.

MATERIAL AND METHOD

Cell lines

MDA-MB-231 cells (ATCC, Rockville, MD, USA), the TNBC cell line, were mainly used in this study. A subline derived from the MDA-MB-231 parental cell was generated using CRISPR/Cas9 methods as described before (Komalasari, et al., 2023). The clone number 22 from gRNA two was then selected based on the protein expression, which was further named MDA-MB-231 LOXL4 knockout (KO) #2-

22 for further experiments. Cells were maintained in DMEM/F12 medium (Thermo Fisher Scientific, Waltham, MA) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA), cultivated mainly in a 10 cm dish, and incubated at 37ºC with 5% CO_2.

In vitro cell analysis

MDA-MB-231 parental and subline LOXL4 KO #2-22 were analyzed using colony formation assay (CFA) to observe the potential of cells to build a colony from a single cell without nudging the neighboring cells. 1 x 10^2 cells were planted in a 60 mm dish. Three dishes were prepared for each parental and LOXL4 KO #2-22 group (n=3) and then incubated for seven days. After colonies formed, 70% EtOH was used for fixation, followed by Crystal Violet staining. One colony was defined as more than or equal to 20 cancer cells (Hirabayashi, et al., 2023).

In vivo animal model and histology specimen

Ten female BALB/c nude mice were prepared and randomly separated into two groups, five mice per group. The mice in the first group were inoculated with 5x10^5 cells of MDA-MB-231 parental into the right mammary fat pad. The mice in the second group were inoculated with MDA-MB-231 LOXL4 KO #2-22 using the same number of cells. Tumor volume was measured after the tumor formed. After 40 days of incubation, tumors were then isolated, fixed with 4% paraformaldehyde (PFA), and then preserved in a frozen tumor block. Histological specimens were obtained from the frozen slices and stained with Masson's Trichrome, Hematoxylin, and Eosin (H&E). Collagen from the Masson 's Trichrome staining was quantified using a Keyence BZ-X analyzer software to detect collagen fibers in the randomly selected slide.

Statistical analysis

Statistical tests were performed in Microsoft Excel for Mac version 16.81. Data were presented in mean and SD. Based on the Student t-test, $p<0.05$ was considered statistically significant.

RESULT

High LOXL4 expression in TNBC drove our attention to the importance of this gene in breast cancer progression. To investigate further this role, the LOXL4 gene was depleted from the MDA-MB-231 parental cells. The establishment of LOXL4 KO #2-22 has been shown in our previous study Komalasari, et al., (2023), the clone was determined based on the protein expression. To assert the concomitant of LOXL4 KO #2-22 in breast cancer cell proliferation, cells were equally spread in low numbers, and then the colony developed from each single cell was observed. After the designated observation time, the size of the colony number formed in the MDA-MB-231 parental group was 51±8.18, and the LOXL4 KO #2-22 group showed 35±3.6 (Figure 1). The number of colonies was significantly reduced ($p<0.05$).

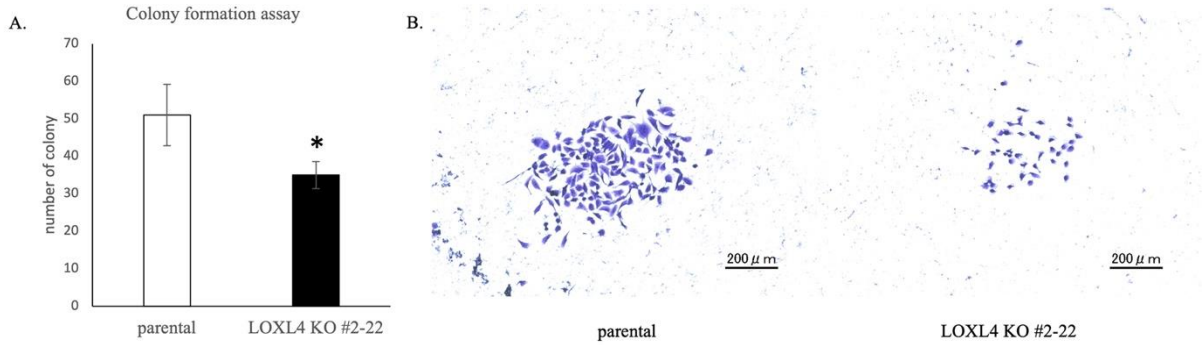

Figure 1. Depletion of LOXL4 affects the single-cell potential to form a colony. (A) Colony number count. (B) Representative of colony size from each group after seven days of incubation.

According to our previous study, the primary tumor growth in the LOXL4 KO group was stalled (Komalasari, et al., 2023). The primary tumor from the breast cancer model was then investigated histologically. The first staining showed a packed and dense configuration of tumor cells in the parental group with dense and regular stroma (Figure 2). LOX family is considered to exert function in the maturation of collagen. To assess the shared function as other LOX family members in the extracellular matrix, i.e., collagen configuration, tumor sections were stained with Masson's Trichrome. Quantification of Masson's Trichrome staining exhibited decreased collagen fibers in the MDA-MB-231 LOXL4 KO #2-22 (21.4±3.6) subline group compared to the parental group (29.5±2.3) with the $p<0.01$ (Figure 3).

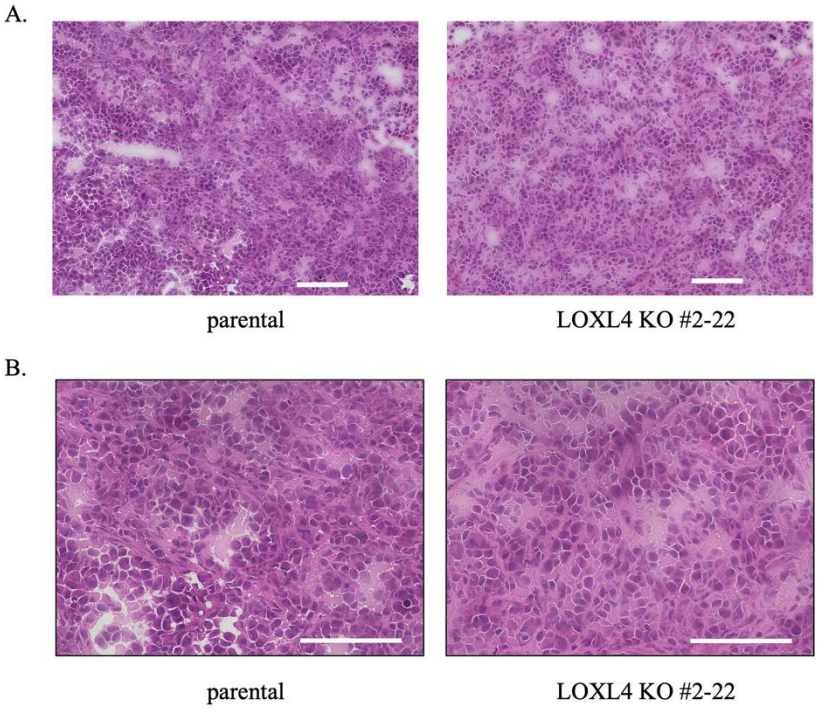

Figure 2. (A) The tumor section from the parental group shows densely packed cancer cells. (B) Larger magnification. The white scale indicates 100 µm.

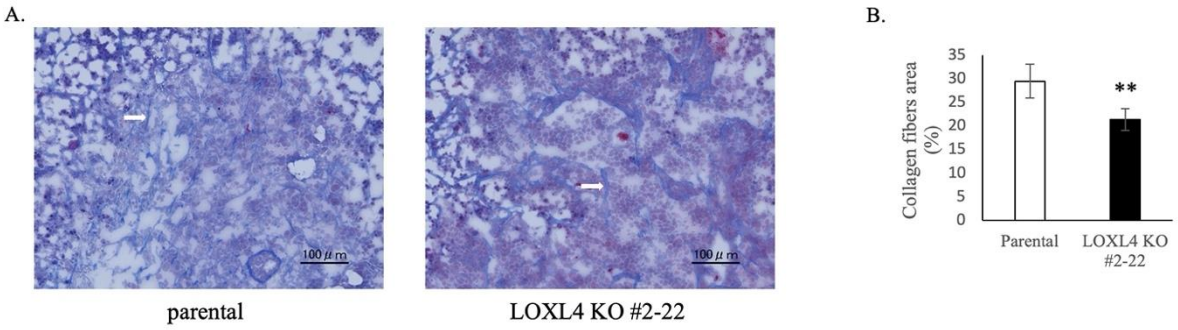

Figure 3. Collagen fibers with Masson's Trichrome staining from tumor section. (A) Representative of Masson's Trichrome staining. The white arrows indicate collagen fibers. (B) Quantification of collagen fibers ($p<0.01$). Scale: 100 µm.

DISCUSSION

In our first screening, we found a high expression of LOXL4 in TNBC, which was not observed in non-TNBC cell lines. To confirm this function, CFA was conducted to evaluate the impact of LOXL4

depletion. Depletion of LOXL4 attenuated the number and size of colonies in the LOXL4 KO #2-22 group, thus reducing cell-cell interaction. Yin, et al., (2020) also showed depressed proliferation of cancer cells on shRNA LOXL4 treatment. This result suggests that LOXL4, other than the ECM, exerts an atypical function in the cancer cell cycle. Additionally, low cell numbers reduce cell-cell interactions, thus preventing downstream mechanisms from mutually inducing cell proliferation. The capability of a single cell to form a colony is also used as a cancer cell stemness indicator (Rajendran & Jain, 2018). Ohta, et al., (2022) observed that slow-proliferated cancer cells have characteristics of stem cells.

After being inoculated orthotopically, the LOXL4 KO #2-22 group developed a smaller tumor size (Komalasari, et al., 2023). Staining of the LOXL4 KO #2-22 tumor showed less ECM with misshapen collagen fibers. LOX family members have been known to partake in ECM maturation which contributed to tumor progression (Maller, et al., 2021). LOXL4 was reported to be associated with collagen I, collagen IV, and liver fibrosis progression (Chen, et al., 2020; Tan, et al., 2021). Thus, LOXL4 supports cancer progression through a common function of LOX family members. Li, et al., (2019) introduce that exosomes in hepatocellular carcinoma (HCC) contain LOXL4 which drives invasion and metastasis. The stiff matrix also contributed to the release of exosomes (Wu, et al., 2023). Therefore, several mechanisms of LOXL4 to induce cancer progression are proposed.

Our previous investigation revealed the surface binding protein of LOXL4, annexin A2 (Komalasari, et al., 2023). This study showed that depletion of LOXL4 reduced collagen deposition, suggesting that secreted LOXL4 affects both neighboring cancer cells and their tumor microenvironment. The impact of LOXL4 in other aspects of TME, including matrix metalloproteinase (MMP) regulation, immune cells, and cancer-associated fibroblast, is also essential to be investigated. Based on current *in vitro* investigation, other intracellular functions of LOXL4 are our next concern. The stemness characteristic of LOXL4 KO #2-22 is an intriguing topic to be explored further.

Strength and limitations

This study supports the concept that LOXL4 has the potential for other functions in breast cancer progression; thus, it is highly expressed in highly invasive cancer cell lines. Our current study was limited to the histological approach of collagen in animal breast cancer models. Further studies are required to disentangle the function of the LOXL4 animal model related to the immune system, matrix metalloproteinase (MMP) regulation, and cancer-associated fibroblast.

CONCLUSION

Our *in vitro* study revealed that depletion of LOXL4 attenuates the ability of a single cell to form a colony. Furthermore, the depletion of LOXL4 decreased collagen from the histological examination in the *in vivo* breast cancer model. Based on these data, the depletion of LOXL4 attenuates the breast cancer model progression. This might be an insight to investigate LOXL4 as a potential target treatment in stalling breast cancer progression.

Acknowledgment

The authors would like to express gratitude to the Japanese Government for the scholarship and support during the study of NLGYK, to all members of the Cell Biology Department, Graduate School of Medicine, Dentistry, and Pharmaceutical Science, Okayama University, and members of the Histology Department, Faculty of Medicine, Udayana University for all their attention and support during the experiment, data collection, and manuscript preparation.

Conflict of Interest

All authors have no conflict of interest.

Ethic Consideration

The animal study was approved by Okayama University with approval number OKU-2020001 on 01-04-2020.

Funding Disclosure

This research was funded by JSPS KAKENHI grant no 20H03516 to M.S.

Author Contribution

NLGYK contributed to the conception and design, collecting analysis and interpretation of the data, drafting the article, and statistical analysis. IGHG drafted the article, performed critical revisions, and provided administrative support. IGNSW contributed to critical revision and administrative support. NT helped with data collection and interpretation and technical support. MS contributed to the conception, drafting of the article, critical revision, and obtaining funding. All authors agreed with the final approval of the article.

REFERENCES

Brassart-Pasco, S., Brézillon, S., Brassart, B., et al. 2020. Tumor microenvironment: Extracellular matrix alterations influence tumor progression. Frontiers in Oncology, 10. doi: 10.3389/fonc.2020.00397.

Chen, W., Yang, A., Jia, J., et al. 2020. Lysyl oxidase (LOX) family members: Rationale and their potential as therapeutic targets for liver fibrosis. Hepatology, 72(2): 729–741. doi: 10.1002/hep.31236.

Choi, S. K. Kim, H. S., Jin, T., et al. 2017. LOXL4 knockdown enhances tumor growth and lung metastasis through collagen-dependent extracellular matrix changes in triple-negative breast cancer. Oncotarget, 8(7): 11977–11989. doi: 10.18632/oncotarget.14450.

Grau-Bové, X., Ruiz-Trillo, I., Rodriguez-Pascual, F. 2015. Origin and evolution of lysyl oxidases. Scientific Reports, 5(1): 10568. doi: 10.1038/srep10568.

Hirabayashi, D., Yamamoto, K. I., Maruyama, A., et al. 2023. LOXL1 and LOXL4 are novel target genes of the Zn2+-bound form of ZEB1 and play a crucial role in the acceleration of invasive events in triple-negative breast cancer cells. Frontiers in Oncology, 13. doi: 10.3389/fonc.2023.1142886.

Hong, X., Yu, J. 2019. Silencing of lysyl oxidase-like 2 inhibits the migration, invasion and epithelial-to-mesenchymal transition of renal cell carcinoma cells through the Src/FAK signaling pathway. International Journal of Oncology. doi: 10.3892/ijo.2019.4726.

Huang, J., Ngai, C. H., Deng, Y., et al. 2022. Cancer incidence and mortality in Asian countries: A trend analysis. Cancer Control, 29: 107327482210959. doi: 10.1177/10732748221095955.

Komalasari, N. L. G. Y., Tomonobu, N., Kinoshita, R., et al. 2023. Lysyl oxidase-like 4 exerts an atypical role in breast cancer progression that is dependent on the enzymatic activity that targets the cell-surface annexin A2. Frontiers in Oncology, 13. doi: 10.3389/fonc.2023.1142907.

Koorman, T., Jansen, K. A., Khalil, A., et al. 2022. Spatial collagen stiffening promotes collective breast cancer cell invasion by reinforcing extracellular matrix alignment. Oncogene, 41(17): 2458–2469. doi: 10.1038/s41388-022-02258-1.

Li, R., Wang, Y., Zhang, X., et al. 2019. Exosome-mediated secretion of LOXL4 promotes hepatocellular carcinoma cell invasion and metastasis. Molecular Cancer, 18(1): 18. doi: 10.1186/s12943-019-0948-8.

Lim, Y. X., Lim, Z. L., Ho, P. J., et al. 2022. Breast cancer in Asia: Incidence, mortality, early detection, mammography programs, and risk-based screening initiatives. Cancers, 14(17): 4218. doi: 10.3390/cancers14174218.

Liu, Y., Liu, X., Chen, F., et al. 2023. LOXL2 promotes tumor proliferation and metastasis by FAK/Src signaling in esophageal squamous cell carcinoma. Electronic Journal of Biotechnology, 63: 18–28. doi: 10.1016/j.ejbt.2023.01.002.

Maller, O., Drain, A. P., Barrett, A. S., et al. 2021. Tumor-associated macrophages drive stromal cell-dependent collagen crosslinking and stiffening to promote breast cancer aggression. Nature Materials, 20(4): 548–559. doi: 10.1038/s41563-020-00849-5.

Microsoft Corporation. 2024. Microsoft Excel for Mac. Available at: https://learn.microsoft.com/en-us/officeupdates/update-history-office-for-mac.

Ohta, Y., Fuji, M., Takahashi, S., et al. 2022. Cell–The cell-matrix interface regulates dormancy in human colon cancer stem cells. Nature, 608(7924): 784–794. doi: 10.1038/s41586-022-05043-y.

Oldfield, R. N., Johnston, K. A., Limones, J., et al. 2018. Identification of Histidine 303 as the catalytic base of lysyl oxidase via site-directed mutagenesis. The Protein Journal, 37(1): 47–57. doi: 10.1007/s10930-017-9749-3.

Rajappa, S., Singh, M., Uehara, R., et al. 2023. Cancer incidence and mortality trends in Asia based on regions and human development index levels: An analyses from GLOBOCAN 2020. Current Medical Research and Opinion, 39(8): 1127–1137. doi: 10.1080/03007995.2023.2231761.

Rajendran, V., Jain, M. V. 2018. *In vitro* tumorigenic assay: Colony forming assay for cancer stem cells: 89–95. doi: 10.1007/978-1-4939-7401-6_8.

Reynaud, C., Ferreras, L., Di Mauro, P., et al. 2017. Lysyl oxidase is a strong determinant of tumor cell colonization in bone. Cancer Research, 77(2): 268–278. doi: 10.1158/0008-5472.CAN-15-2621.

Shao, J., Lu, J., Zhu, W., et al. 2019. Derepression of LOXL4 inhibits liver cancer growth by reactivating compromised p53. Cell Death & Differentiation, 26(11): 2237–2252. doi: 10.1038/s41418-019-0293-x.

Sharma, R., Abbastabar, H., Abdulah, D. M., et al. 2024. Temporal patterns of cancer burden in Asia, 1990–2019: A systematic examination for the Global Burden of Disease 2019 study. The Lancet Regional Health - Southeast Asia, 21: 100333. doi: 10.1016/j.lansea.2023.100333.

Tan, H., Wang, N., Zhang, C., et al. 2021. Lysyl oxidase-like 4 fosters an immunosuppressive microenvironment during hepatocarcinogenesis. Hepatology, 73(6): 2326–2341. doi: 10.1002/hep.31600.

Vallet, S. D., Ricard-Blum, S. 2019. Lysyl oxidases: From enzyme activity to extracellular matrix cross-links. Essays in Biochemistry. Edited by J. Adams, 63(3): 349–364. doi: 10.1042/EBC20180050.

Winkler, J., Abisoye-Ogunniyan, A., Metcalf, K. J., et al. 2020. Concepts of extracellular matrix remodelling in tumour progression and metastasis. Nature Communications, 11(1): 5120. doi: 10.1038/s41467-020-18794-x.

World Health Organization (2024) Breast cancer, WHO. Available at: https://www.who.int/news-room/fact-sheets/detail/breast-cancer.

Wu, B., Liu, D., Guan, L., et al. 2023. Stiff matrix induces exosome secretion to promote tumour growth. Nature Cell Biology, 25(3): 415–424. doi: 10.1038/s41556-023-01092-1.

Yin, H., Wang, Y., Wu, Y., et al. 2020. EZH2-mediated epigenetic silencing of miR-29/miR-30 targets LOXL4 and contributes to tumorigenesis, metastasis, and immune microenvironment remodeling in breast cancer. Theranostics, 10(19): 8494–8512. doi: 10.7150/thno.44849.

ORIGINAL RESEARCH REPORT

Age, Sex, and Types of Occupation with Histopathological Types in Patients with Ocular Surface Squamous Neoplasia (OSSN) in a Tertiary Hospital in Surabaya, Indonesia

Stella Agatha Widjaja[1], Delfitri Lutfi[2*], Linda Dewanti[3], Alphania Rahniayu[4], Fitria Kusumastuti[5]

[1]Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.
[2]Department of Ophthalmology, Faculty of Medicine, Universitas Airlangga; Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.
[3]Department of Public Health and Preventive Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.
[4]Department of Anatomy Pathology, Faculty of Medicine, Universitas Airlangga; Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.
[5]East Java Community Eye Hospital, Surabaya, Indonesia.

Article Info

Article history:
Received 24-12-2023
Revised 04-03-2024
Accepted 17-04-2024
Published 01-07-2024

Keywords:
Ocular surface squamous neoplasia
Ocular tumor
Cancer
Public health

***Corresponding author**:
Delfitri Lutfi
delfitri-l@fk.unair.ac.id

ABSTRACT

Background: Ocular Surface Squamous Neoplasia (OSSN) is a spectrum of tumors affecting the conjunctiva and cornea, in which a more invasive type of OSSN has a higher recurrence rate. As a tropical country, Indonesia is constantly exposed to intense ultraviolet (UV) radiation, the main risk factor for OSSN. Despite this, there are very few studies regarding OSSN in Indonesia. **Objective**: This study aimed to analyze the association between sociodemographic characteristics such as age, sex, and types of occupation (outdoor and indoor) and histopathological types of OSSN to fill the gap in research and contribute to early diagnostic strategies. **Material and Method**: Sociodemographic and histopathological data were extracted from the medical records of patients who were histopathologically diagnosed with OSSN at Dr. Soetomo General Hospital from 2017 to 2021. Data from 88 samples that met the inclusion and exclusion criteria were classified and analyzed with descriptive statistics and a two-sided Fisher's exact test using SPSS version 27.0 (IBM Corp., Armonk, N.Y., USA). **Result**: The exact test results of the two-sided Fisher's exact test revealed a statistically significant relationship between age ($p = 0.0004$) and occupation type ($p = 0.049$) with histopathological types of OSSN. However, no significant association was found between sex and histopathological types of OSSN ($p = 0.130$). **Conclusion**: Age and occupation were found to have a significant association with the histopathological types of OSSN, reinforcing the need for further exploration of their strength and nature to be considered by future researchers.

How to cite:
Widjaja, S.A., Lutfi, D., Dewanti, L., et al. 2024. Age, Sex, and Types of Occupation with Histopathological Types in Patients with Ocular Surface Squamous Neoplasia (OSSN) in a Tertiary Hospital in Surabaya, Indonesia. Majalah Biomorfologi-Biomorphology Journal, 34(2):74-82.

Majalah Biomorfologi (Biomorphology Journal) p.ISSN:0215-8833, e.ISSN: 2716-0920
doi: 10.20473/mbiom.v34i2.2024.74-82

Highlights

1. The histopathological types of OSSN were significantly associated with age and occupation.
2. The study's findings enhance the understanding of OSSN in Indonesia and emphasize the importance of UV exposure, occupation, and age in its development.

BACKGROUND

Indonesia has a tropical environment and receives significant sunlight, resulting in high amounts of UV radiation. The UV index in Indonesia ranges from 9 to 11+, making it one of the countries with the highest UV indices worldwide (Istirohah, et al., 2018). Ocular Surface Squamous Neoplasia (OSSN) is a prevalent ocular surface tumor linked primarily to UV exposure, with additional risk factors including age, gender, immunosuppression, HPV infection, and insufficiency of vitamin A (Dandala, 2015; Höllhumer, et al., 2020; Gurnani & Kaur, 2023). However, OSSN demographics vary regionally, prompting a need for specific investigations in Indonesia (Höllhumer, et al., 2020).

Age, a key factor, is associated with increased OSSN risk, especially in individuals aged 40 and older (Dandala, 2015). Gender predispositions vary globally, with OSSN being predominantly male in some regions and predominantly female in others (Tananuvat, et al., 2022). Understanding OSSN's gender predisposition is crucial for assessing its impact in Indonesia.

Occupational exposure to solar radiation is a significant hazard for the approximately 40 million Indonesians working in agriculture, forestry, and fisheries (Statistics Indonesia, 2023). Studies reveal a higher OSSN incidence among outdoor occupations; however, the importance of the relationship varies (Dandala, 2015; Gichuhi, et al., 2016). The frequency of outdoor employment in Indonesia emphasizes the urgency of examining OSSN as a severe hazard.

Diagnosing OSSN based on clinical features alone is challenging. Hence, histopathology is required to confirm the diagnosis and determine the prognosis. OSSN includes dysplasia, carcinoma in situ, and invasive squamous cell carcinoma (Mishra, et al., 2022). Early detection and treatment are crucial for reducing recurrences, and understanding the association between demographic factors and histopathological types can contribute to a more effective diagnosis strategy (Cicinelli, et al., 2018).

Despite the substantial risk OSSN poses in Indonesia, there is a notable gap in research specifically addressing its association with histopathological types. This study aimed to fill this gap by analyzing medical records from a tertiary hospital of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, from 2017 to 2021, assessing the relationship between age, sex, occupation, and histopathological types of OSSN.

OBJECTIVE

The purpose of this study was to describe the distribution of histopathological types of OSSN among diagnosed patients, to investigate the distribution of age, biological sex, and occupation types within this patient group, and to conduct analyses to determine associations between age, biological sex, occupation type, and histopathological types among OSSN patients.

MATERIAL AND METHOD

This was an analytic cross-sectional study that examined the medical records of patients who were clinically diagnosed with Ocular Surface Squamous Neoplasia (OSSN) at the Department of Ocular Oncology of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, between January 2017 and December 2021. The study employed a whole population sample method, where all patients were included if they met the specified criteria. The inclusion criteria required patients to have histological data. In contrast, the exclusion criteria included those who previously had unrelated eye procedures, chronic eye disorders, recurrent OSSN, or incomplete records. Data were collected from July to August 2023, and 88 patients were examined. This study received ethical clearance from the Ethical Committee

of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (no. 2276/113/4/VII/2023) on 25-07-2023.

The retrieved characteristics included age, sex, occupation, and histological type. For analytical purposes, age was categorized as 0-39 and ≥40, occupation was classified as outdoor and indoor, and histopathological type was categorized as non-invasive (including mild-moderate dysplasia, moderate-severe dysplasia, severe dysplasia, and carcinoma in situ) and invasive (squamous cell carcinoma). The categorization of age groups and histological types was conducted based on a previous study by Dandala, (2015). The classification of occupations was performed by applying the methodology utilized in the study conducted by Cox-Ganser & Henneberger (2021). The classification was done by first matching the occupations as written in medical records to their equivalent Standard Occupational Classification (SOC) code(s) (U.S. Bureau of Labor Statistics, 2018). Afterward, the occupations were classified into outdoor and indoor work based on data extracted from the Occupational Information Network (O*NET) (Donsbach, et al., 2003). This data was the scored answer to the question: *How often does your current job require you to work outdoors, exposed to all weather conditions?* Occupations with an average score of ≥75 (once a week to every day) were categorized into outdoor work.

Table 1. Classification of patients into outdoor and indoor workers (Donsbach, et al., 2003; U.S. Bureau of Labor Statistics, 2018).

Occupation	Occupation code(s)	Score	Occupation type	n
Farmer	45-2090	82.3	Outdoor	27
Fisher	45-3030	95	Outdoor	1
Military/Police Officer	33-3050	91	Outdoor	2
Driver	53-3030	91	Outdoor	1
Field employee	45-2090	82.3	Outdoor	2
Welder	51-4120	21	Indoor	2
Private employee	43-0000	13.6	Indoor	13
Civil servant	43-0000	13.6	Indoor	5
Teacher	25-1000, 25-2000	15.2	Indoor	2
Student	25-1000, 25-2000	15.2	Indoor	6
Housewife	39-9011.00, 39-9011.01, 35-9021.00, 37-1011.00, 37-2012.00, 35-2013.00, 21-1021.00	35.9	Indoor	19
Entrepreneur	11-1011.00, 11-1021.00, 13-1023.00, 13-1161.00, 41-1010	27.7	Indoor	6
Chef	35-1011	36	Indoor	1
Factory worker	11-3051	33	Indoor	1

Table 1 shows that farmers, soldiers or police officers, drivers, and field employees were classified as outdoor workers, while welders, private employees, civil servants, teachers, students, housewives, entrepreneurs, chefs, and factory workers were classified as indoor workers. As housewives did not have a SOC code, several occupations with similar activities were substituted. As students did not have a SOC code, we substituted them using data from teachers, as they shared identical activities at school.

The data were analyzed with descriptive statistics to determine the distribution of age, sex, occupation types, and histopathological types among patients with OSSN. The data were also analyzed using the two-sided Fisher's Exact test to determine the association between age, biological sex, occupation types, and the histopathological types of OSSN. The statistical analysis was performed using IBM SPSS Statistics for Windows, version 27.0 (IBM Corp., Armonk, N.Y., USA).

RESULT

Table 2 illustrates the distribution of age, sex, occupation type, and histopathological type. The mean age of patients is 52.7±19.1, with the majority falling into the 40 and older age group (75%), as categorized based on a previous study (Dandala, 2015). The study population was predominantly male, with 52 male patients (59.1%) compared to 36 female patients (40.9%). There were 55 indoor workers

(62.5%) and 33 outdoor workers (37.5%) among the patients. The most common histopathological type was conjunctival intraepithelial neoplasia (48.9%), followed by squamous cell carcinoma (46.6%) and carcinoma in situ (4.5%). For analytical purposes, we classified our population's histopathological types of OSSN into non-invasive and invasive OSSN. Non-invasive OSSN refers to conjunctival intraepithelial neoplasia and carcinoma in situ, while invasive OSSN refers to squamous cell carcinoma. This was done due to the low count of CIS, which hindered the application of Fisher's exact test. Most patients presented with a non-invasive OSSN (53.4%) rather than an invasive (46.6%).

Table 2. Distribution of age, sex, occupation type, and histopathological type. Processed from research data.

Variable	n = 88	%
Age		
0-39	22	25
≥40	66	75
Sex		
Male	52	59.1
Female	36	40.9
Occupation type		
Outdoor	33	37.5
Indoor	55	62.5
Histopathological type		
Non-invasive	47	53.4
Conjunctival Intraepithelial Neoplasia	43	48.9
Carcinoma in situ	4	4.5
Invasive	41	46.6
Squamous cell carcinoma		

We found a significant relationship between age (p = 0.0004) and occupation type (p = 0.049) with histopathological types of OSSN, as seen in Table 3. However, there was no significant association between sex and histopathological type (p = 0.130). Most patients with invasive OSSN are 40 years older (92.7%). Within 22 patients younger than 40, a majority (19) presented with non-invasive OSSN. Patients with non-invasive OSSN had an equal sex distribution, consisting of 24 males (51.1%) and 23 females (48.9%). Meanwhile, patients with invasive OSSN were predominantly male (68.3%). While most patients were indoor workers, we could observe that among outdoor workers, invasive OSSN was the most prevalent type (20 out of 33).

Table 3. Analysis result of two-sided fisher's exact test.

Parameter	Non-invasive	Invasive	p-value
Age			
0-39	19 (40.4%)	3 (7.3%)	0.0004
≥40	28 (59.6%)	38 (92.7%)	
Sex			
Male	24 (51.1%)	28 (68.3%)	0.130
Female	23 (48.9%)	13 (31.7%)	
Occupation type			0.049
Outdoor	13 (27.7%)	20 (48.8%)	
Indoor	34 (72.3%)	21 (51.2%)	

DISCUSSION

Distribution of age, sex, occupation type, and histopathological type

This study categorized the population into ages 0-39 and ≥40 to satisfy Fisher's Exact test conditions. A previous study conducted in India found 65 patients (57.5%) aged ≥40 and 48 patients (42.5%) aged

39 and younger (Dandala, 2015). When compared, there was a more significant proportion of middle-aged and older patients in this study (75%).

The ratio of males to females in patients with OSSN differs based on region. A systematic review revealed that most OSSN patients in temperate climates are older males, while OSSN patients in tropical climates, such as Eastern and Southern Africa, are typically young women (Gichuhi, et al., 2013). Based on Table 3, this study found more cases of OSSN in males (59.1%) rather than females (40.9%), aligning with other Indonesian studies where 62.5%-70% of OSSN patients are male (Ardjaja, 2017; Sandraningrum & Dahlan, 2019). Despite being a tropical country, Indonesia's OSSN gender distribution is different from the trend seen in Eastern and Southern Africa, where women make up 50%-70% of patients with OSSN. Africa's higher HIV rates in females might explain their higher OSSN incidence (Gichuhi, et al., 2014; Höllhumer, et al., 2023).

One likely explanation behind this discrepancy is the variation in HIV prevalence between the studies' populations. The prevalence of HIV in Eastern and Southern African countries is comparably higher than the prevalence of HIV in Indonesia (0.4%) (International Labour Organization, 2010). In Africa, HIV infection is more often found in females rather than males, which may explain why the sex predisposition in their studies lean towards females (Gichuhi, et al., 2014; Höllhumer, et al., 2023).

The most prominent risk factor for OSSN is exposure to UV light (Gichuhi, et al., 2016). Various factors affect a person's cumulative UV exposure, such as individual, environmental, and occupational factors (Modenese, et al., 2018). Some occupations involve more significant exposure to UV radiation due to the nature of their job requirements. Based on Table 1, most patients with OSSN are farmers, followed by housewives and private employees.

Farmers typically spend significant time outdoors, exposing them to direct sunlight for extended periods and during daylight hours when UV radiation is strongest. Their cumulative UV exposure is influenced by their duration of work, at what time they work, their environment's surface, and their sun-protective behavior (Modenese, et al., 2018).

A housewife is a woman whose job is to manage the household by doing tasks such as cooking, cleaning, and childcare and who does not have paid employment. There are 19 housewives (21.6%) found in this study, making it the second most common occupation among the study population. One plausible explanation is that many activities done by housewives require them to be outside, for example gardening, hanging the laundry, shopping in open-air markets, tending the yard, cleaning the exterior of the house, or they could be outside for transportation (Tesfai, et al., 2021). Other than that, they may also have a hobby requiring them to spend much time outside. There is also a possibility that they had a different occupation before their hospital visit, which was how they were exposed to UV. Nevertheless, as these were not specified in our analyzed medical records, we cannot confirm these possibilities.

Private employees are any people who work for a privately owned company. In this study, private employees were also found in great numbers compared to the total population, with 13 patients (14.8%). Initially, our database classified 20 patients classified as private employees. Upon manual inspection, we found that two patients worked as welders, one as a farmer, one as a chef, one as a factory worker, and two as field employees. This shows that the definition of private employee is comprehensive, therefore making it difficult to classify them as an indoor or outdoor occupation. Out of 20 private employees, only seven were described clearly, while 13 patients did not have a specific description of their occupation written in the medical records. There is a possibility that some of these 13 patients may have worked outdoors, and the lack of specificity is a limitation that we acknowledge.

Upon classification into outdoor and indoor occupations, as seen in Table 1, 62.5% of patients work indoors, while 37.5% work outdoors. Although classified by different methods, this study's finding was similar to a previous study where 70% were involved in indoor and 30% in outdoor occupations (Meel, et al., 2017). However, this distribution is not aligned with the fact that outdoor occupation has been recognized as a risk factor for OSSN in various studies (Gichuhi, et al., 2013; Dandala, 2015).

Among 88 patients histopathologically diagnosed with OSSN, most patients presented with CIN (48.9%), followed by SCC (46.6%) and CIS (4.5%). This corresponds with prior studies done in Bandung and Denpasar, where CIN (41.5%-50%) is the most common histopathological finding in patients with OSSN, followed very closely by SCC (39%-40%), then by CIS (10%-19.5%) (Ardjaja, 2017; Sandraningrum & Dahlan, 2019). These findings contrasted with studies in Nigeria and Kenya, where invasive SCC is the most frequently diagnosed lesion (58.8%-60.3%) (Gichuhi, et al., 2015;

Lomelí-Linares, et al., 2020). This variation suggests the influence of geographical factors such as climate, altitude, latitude, and ozone concentrations (Modenese, et al., 2018).

Another stark difference between Nigeria, Kenya, and Indonesia is the proportion of HIV-positive patients, with HIV being a predictive factor of higher-grade OSSN. The higher HIV prevalence in African countries could contribute to the observed histopathological variations, underscoring the multifactorial nature of OSSN (Rathi, et al., 2018; Suleiman, et al., 2022).

In this study, 53.4% of patients had a non-invasive OSSN, while 46.6% had an invasive OSSN. This study's population had a higher percentage of invasive OSSN cases compared to a previous study by Dandala (2015), where invasive OSSN only comprised 35.4% of the population.

Association between Age, Sex, and Types of Occupation with Histopathological Type of OSSN

This study investigated the association between age and the histopathological types of OSSN. While previous studies have yet to support a precise relationship between age and histopathological types, existing studies have proven that age plays a significant role in the development of OSSN (Dandala, 2015; Gurnani & Kaur, 2023).

This study found a significant association between age and histopathological type ($p = 0.0004$). Table 3 shows that adults aged 40 and older have a higher tendency to present with invasive OSSN compared to the younger group. Older adults have been exposed to UV light for longer, thus having higher cumulative UV exposure than younger groups. Older adults are also generally more susceptible to the development of malignancy due to the deterioration of their immune system (D'Orazio, et al., 2013). Meanwhile, many middle-aged adults were also found to have a more malignant type of OSSN, possibly because UV radiation has become more intense over time due to ozone depletion and the increasing occurrence of extreme climate events (Barnes, et al., 2023). Due to the increased intensity, the cumulative UV exposure in middle-aged adults may be comparable to older adults even though they have been exposed to UV for a shorter time.

Our study found no statistically significant association between biological sex and histopathological type ($p = 0.130$), aligning with a previous study which did not find any statistically significant association between sex and OSSN (Smith, et al., 2019). This study's findings also aligned with a study that found no statistically significant difference between females' and males' incidence of OSSN in Asia, even though a significant difference was found in other regions such as Central America, South America, North America, and Europe (Gichuhi, et al., 2013).

In contrast, a previous study done in Nigeria found that male sex is associated with higher-grade lesions of OSSN ($p = 0.02$). Several factors may contribute to the discrepancy between this study and the previous study. One possible explanation is the genetic and environmental differences in the studies' populations. While Nigeria and Indonesia share similar climates, geographical features, and certain sociodemographic factors, the study populations are composed differently. Nigeria, with its predominantly black African population, exhibits a different genetic constitution compared to the Asian population found in Indonesia. Genetic factors could potentially contribute to susceptibility to OSSN. However, there has not been any study that could prove a significant association between race and ocular surface squamous neoplasia as of date. Genetic factors' impact on gender-related variations in OSSN remains an area for further study (Suleiman, et al., 2022).

Additionally, the disparity observed in our study results may be attributed to an inherent selection bias, given that both studies were done in single institutes, the Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, and Ahmadu Bello University Teaching Hospital, respectively. The difference in referral patterns and accessibility and the limited pool of patients in both institutions were some underlying reasons for selection bias in single-institution-based studies. As this study was the first to be done in Indonesia, there was limited basis for direct comparison to prior research.

Table 3 shows the distribution of outdoor and indoor workers across different histopathological types. Most outdoor workers (20 out of 33) have been diagnosed with invasive OSSN, while 34 out of 55 indoor workers are diagnosed with non-invasive OSSN. From this finding, we can observe that most patients who work outdoors have developed a more malignant lesion, possibly due to SCC's strong association with UV radiation as found in a study by Gichuhi, et al., (2013).

When the association between occupation types and histopathological types was analyzed, this study found a statistically significant relationship between the two variables ($p = 0.049$). This finding agrees with a previous study which found an association, albeit weak, between outdoor occupations and OSSN

(Gichuhi, et al., 2013).

Strength and limitations

This study was conducted first in Indonesia, a tropical country susceptible to OSSN. Its limitations include its reliance on medical records, which lacks data on potential confounding variables, such as HIV history, smoking habits, outdoor leisure activities, hobbies, and sun-protective habits, which poses a significant limitation. The use of BLS and O*NET for occupation classification also has limitations, with certain worker types not represented in the databases and potential discrepancies between American and Indonesian work environments.

To enhance research data quality, the hospital can improve its medical record system by incorporating additional patient information, including HIV status, smoking habits, detailed job descriptions, leisure activities, and sun-protective behaviors. Future researchers may also consider using a questionnaire to collect data for potential occupational hazards, protective habits, daily work duration, and job tenure. Collecting this information through patient questionnaires could contribute to a more comprehensive and reliable occupational classification system.

CONCLUSION

Most patients diagnosed with ocular surface squamous neoplasia (OSSN) at Dr. Soetomo General Hospital histopathologically exhibited non-invasive OSSN in males aged 40 and older, and worked indoors. Furthermore, we identified statistically significant associations between age and occupation with histopathological type of OSSN. Although these associations' specific direction and strength were not determined in this study, the higher prevalence of invasive types of OSSN observed among older individuals and outdoor workers suggests the need for targeted screening and preventive measures for these high-risk populations. These findings also reinforce the need for future researchers to further explore its strength and nature.

Acknowledgment

The authors acknowledge the contribution of the staff at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, who assisted in conducting this study.

Conflict of Interest

All authors have no conflict of interest.

Ethic Consideration

This study received ethical clearance from the Ethical Committee of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (no. 2276/113/4/VII/2023) on 25-07-2023.

Funding Disclosure

None.

Author Contribution

SAW contributes to the conception and design, analysis and interpretation of the data, drafting of the article, and final approval. DL contributes to the conception and design, drafting of the article, critical revision of the article for important intellectual content, and final approval of the article. LD contributes to the conception and design, drafting of the article, critical revision of the article for important intellectual content, and final approval of the article. AR contributes to the conception and design, drafting of the article, critical revision of the article for important intellectual content, and final approval of the article. FK contributes to the conception and design, drafting of the article, critical revision of the article for important intellectual content, and final approval of the article.

REFERENCES

Ardjaja, Y. K. 2017. Ocular surface squamous neoplasia patient's characteristic at RSUP Sanglah Denpasar 2013-2015. Medicina, 48(3). doi: 10.15562/medicina.v48i3.144.

Barnes, P. W., Robson, T. M., Zepp, R. G., et al. 2023. Interactive effects of changes in UV radiation and climate on terrestrial ecosystems, biogeochemical cycles, and feedbacks to the climate system. Photochemical & Photobiological Sciences, 22(5): 1049–1091. doi: 10.1007/s43630-023-00376-7.

Cicinelli, M. V., Marchese, A., Bandello, F., et al. 2018. Clinical management of ocular surface squamous neoplasia: A review of the current evidence. Ophthalmology and Therapy, 7(2): 247–262. doi: 10.1007/s40123-018-0140-z.

Cox-Ganser, J. M., Henneberger, P. K. 2021. Occupations by proximity and indoor/outdoor work: Relevance to Covid-19 in all workers and black/Hispanic workers. American Journal of Preventive Medicine, 60(5): 621–628. doi: 10.1016/j.amepre.2020.12.016.

D'Orazio, J., Jarrett, S., Amaro-Ortiz, A., et al. 2013. UV radiation and the skin. International Journal of Molecular Sciences, 14(6): 12222–12248. doi: 10.3390/ijms140612222.

Dandala, P. P. 2015. Ocular surface squamous neoplasia (OSSN): A retrospective study. Journal of Clinical and Diagnostic Research, 9(11). doi: 10.7860/JCDR/2015/16207.6791.

Donsbach, J., Tsacoumis, S., Sager, C., et al. 2003. O*NET analyst occupational abilities ratings: Procedures. Suite. Available at: https://www.onetcenter.org/dl_files/AnalystProc.pdf.

Gichuhi, S., Sagoo, M., Weiss, H. A., et al. 2013. Epidemiology of ocular surface squamous neoplasia in Africa. Tropical Medicine & International Health, 18(12): 1424–1443. doi: 10.1111/tmi.12203.

Gichuhi, S., Ohnuma, S., Sagoo, M. S., et al. 2014. Pathophysiology of ocular surface squamous neoplasia. Experimental Eye Research, 129: 172–182. doi: 10.1016/j.exer.2014.10.015.

Gichuhi, S., Macharia, E., Kabiru, J., et al. 2015. Clinical presentation of ocular surface squamous neoplasia in Kenya. JAMA Ophthalmology, 133(11): 1305. doi: 10.1001/jamaophthalmol.2015.3335.

Gichuhi, S., Macharia, E., Kabiru, J., et al. 2016. Risk factors for ocular surface squamous neoplasia in Kenya: A case-control study. Tropical Medicine & International Health, 21(12): 1522–1530. doi: 10.1111/tmi.12792.

Gurnani, B., Kaur, K. 2023. Ocular surface squamous neoplasia. StatPearls. Available at: http://www.ncbi.nlm.nih.gov/pubmed/34262162.

Höllhumer, R., Williams, S., Michelow, P. 2020. Ocular surface squamous neoplasia: Population demographics, pathogenesis, and risk factors. African Vision and Eye Health, 79(1). doi: 10.4102/aveh.v79i1.553.

Höllhumer, R., Michelow, P., Williams, S. 2023. Demographics, clinical presentation and risk factors of ocular surface squamous neoplasia at a Tertiary Hospital, South Africa. Eye, 37(17): 3602–3608. doi: 10.1038/s41433-023-02565-1.

IBM Corp. 2020. IBM SPSS statistics for Windows, version 27.0. Armonk, NY: IBM Corp. Available at: https://www.ibm.com/support/pages/downloading-ibm-spss-statistics-27010.

International Labour Organization. 2010. International Standard Classification of Occupations (ISCO), ILO. Available at: https://www.ilo.org/public/english/bureau/stat/isco/index.htm.

Istirohah, T., Sari, D. M., Jannah, M. A., et al. 2018. Synthesis and bioactivity of TiO 2 /PPy micelle with Pachyrhizus erosus extract as UV absorbent material. Journal of Physics: Conference Series, 1093: 012023. doi: 10.1088/1742-6596/1093/1/012023.

Lomelí-Linares, D., García-Salgado, L., Riancho-Sánchez, G., et al. 2020. Frequency of conjunctival epithelial dysplasia in patients with pterygium. Arquivos Brasileiros de Oftalmologia, 83(4). doi: 10.5935/0004-2749.20200053.

Meel, R., Dhiman, R., Vanathi, M., et al. 2017. Clinicodemographic profile and treatment outcome in patients of ocular surface squamous neoplasia. Indian Journal of Ophthalmology, 65(10): 936. doi: 10.4103/ijo.IJO_251_17.

Mishra, D. K., Taneja, S., Singh, V., et al. 2022. Histopathological spectrum of ocular surface squamous neoplasia: A retrospective study of 776 lesions. Indian Journal of Pathology & Microbiology, 65(1): 3–7. doi: 10.4103/IJPM.IJPM_1313_20.

Modenese, A., Korpinen, L., Gobba, F. 2018. Solar radiation exposure and outdoor work: An underestimated occupational risk. International Journal of Environmental Research and Public Health, 15(10): 2063. doi: 10.3390/ijerph15102063.

Rathi, S. G., Ganguly, A., Kaliki, S. 2018. Ocular surface squamous neoplasia in HIV-infected patients: Current perspectives. HIV/AIDS - Research and Palliative Care, 10: 33–45. doi: 10.2147/HIV.S120517.

Sandraningrum, S., Dahlan, M. R. 2019. Characteristic of ocular surface squamous neoplasia patients in Cicendo Eye Center within January 2012 - June 2014. Ophthalmologica Indonesiana, 43(1): 40. doi: 10.35749/journal.v43i1.136.

Smith, L. M., Lamba, S., Karp, C. L, et al. 2019. Epidemiology of ocular surface squamous neoplasia in Veterans: A retrospective case-control study. Eye and Vision, 6(1): 14. doi: 10.1186/s40662-019-0138-1.

Statistics Indonesia. 2023. Population 15 years and over who work according to main employment 1986 - 2022. Statistics Indonesia. Available at: https://www.bps.go.id/id/statistics-table/1/ \OTcwIzE=/ penduduk-15-tahun-ke-atas-yang-bekerja-menurut-lapangan-pekerjaan-utama-1986---2022.html.

Suleiman, D. E., Liman, A. A., Waziri, G. D., et al. 2022. Clinicopathological characteristics of ocular surface squamous neoplasia: A 10-year review form a referral tertiary center in Nigeria. International Ophthalmology, 42(12): 3905–3911. doi: 10.1007/s10792-022-02411-5.

Tananuvat, N., Niparugs, M., Wiwatwongwana, D., et al. 2022. Ocular surface squamous neoplasia in Northern Thailand: A 16-year review. BMC Ophthalmology, 22(1): 121. doi: 10.1186/s12886-022-02340-y.

Tesfai, B., Kebede, S., Kibreab, F., et al. 2021. Prevalence of Solar Keratopathy, Pterygium and Cataract in the Islands of Northern Red Sea Zone, Eritrea: Cross-sectional study, 2021. Clinical Ophthalmology, 15: 2983–2991. doi: 10.2147/OPTH.S321413.

U.S. Bureau of Labor Statistics. 2018. 2018 Standard Occupational Classification System. U.S. Bureau of Labor Statistics. Available at: https://www.bls.gov/soc/2018/major_groups.htm.

ORIGINAL RESEARCH REPORT

Demographic Characteristics and ABO Blood Group Genotypes Distribution among Sickle Cell Anemia Patients in Birnin Kebbi, Northwestern Nigeria

Tajudeen Olanrewaju Yahaya*, Asiya Koko Attahiru, Abdulgafar Bala Ibrahim, Mutiyat Kehinde Adewale, Adamu Zainab Fari, Abdullahi Saadatu
Department of Biological Sciences, Federal University Birnin Kebbi, Kebbi State, Nigeria.

Article Info

Article history:
Received 24-02-2024
Revised 07-06-2024
Accepted 19-06-2024
Published 01-07-2024

Keywords:
ABO blood group genotypes
Blood group O-
Demographic information
Sickle cell anemia
Human & Health

***Corresponding author**:
Tajudeen Olanrewaju Yahaya
yahaya.tajudeen@fubk.edu.ng

ABSTRACT

Background: Demographic information and ABO blood group genotypes have been shown by studies to be useful tools in the management of sickle cell anemia. Regrettably, there is a shortage of literature on this information in Birnin Kebbi, northwestern Nigeria. **Objective**: This study determined demographic characteristics, ABO blood group genotypes, and sickle cell genotypes of sickle cell anemia patients in Birnin Kebbi. **Material and Method**: A structured checklist was used to collect data from the medical records of 99 patients who attended the Federal Medical Center in the city between November 2022 to November 2023. **Result**: The results revealed that most of the patients fell within the 1 to 10 years age group, comprising 44 individuals (44.4%), and the 11 to 20 years age group, comprising 33 individuals (33.3%). Of the patients, 48 (48.5%) were males, while 51 (51.5%) were females. Patients with ABO blood genotype O^- (33.3%) and A^+ (18.0%) were the most prevalent and least severely affected, while blood groups O^+ (3%) and AB^- (6%) were the least prevalent and most severely affected. Two variants of sickle cells (HbSS and HbSC) were identified, with HbSS (92.9%) being the most prevalent. **Conclusion**: Based on these findings, efforts should be made to ensure an ample blood supply with groups O^-, A, and AB^+ in blood banks. Moreover, studies have shown ABO blood groups to influence responses to diets, so precision medicine tailored to individual patients' blood groups is recommended. Individuals with O^+ and AB^- -blood groups should be given special attention, as they exhibit the severe form of the disease.

How to cite:
Yahaya, T. O., Attahiru, A. K., Ibrahim, A. B., et al. 2024. Demographic Characteristics and ABO Blood Group Genotypes Distribution among Sickle Cell Anemia Patients in Birnin Kebbi, Northwestern Nigeria. Majalah Biomorfologi-Biomorphology Journal, 34(2): 83-92.

Majalah Biomorfologi (Biomorphology Journal) p.ISSN:0215-8833, e.ISSN: 2716-0920
doi: 10.20473/mbiom.v34i2.2024.83-92

Highlights

1. Sickle cell anemia affects both males and females in Birnin Kebbi and is predominant among children between 1 to 10 years of age.
2. It is also more prevalent but less severe among individuals with O- and A+ ABO blood groups in the city.

BACKGROUND

Sickle cell anemia is a monogenic disease characterized by the deformation of red blood cells and variable clinical outcomes (Adigwe, et al., 2023). It results from a point mutation involving base-pair substitution of GAG to GTG in the β-globin gene at chromosome 11, specifically within the 17th nucleotide, where thymine is replaced by adenine (Onoja, et al., 2021; Adigwe, et al., 2023). Consequently, during translation, valine replaces glutamic acid, causing abnormal hemoglobin called hemoglobin S (HbS) (Onoja, et al., 2021; Adigwe, et al., 2023). This mutation causes neighboring molecules of HbS to polymerize upon deoxygenation, resulting in long, stiff concatenations of hemoglobin. This process distorts the biconcave shape of red cells to a sickle shape, along with some other abnormal features (Lu, et al., 2021). The outcome is a viscous red blood cell characterized by suboptimal rheological properties and a tendency to get stuck in microvascular regions (Lu, et al., 2021). The inheritance of sickle cell disease follows Mendelian principles, wherein affected individuals inherit either one (heterozygous AS) or two (homozygous SS) sickle cell genes from their parents (Mbiya, et al., 2020). Homozygous SS (HbSS) is the most severe subtype, symptomized by both acute and chronic complications such as painful events, infections, acute chest syndrome, anemia, eye damage, dactylitis, stroke, splenic sequestration, chronic organ damage with substantial morbidity, early mortality (Abboud, 2020; Katawandja, et al., 2020; Ngwengi, et al., 2020). These symptoms occur in sickle cell patients due to blood cells adhering to the inner walls of the blood vessels in the brain, thereby restricting blood flow (Hussaini, et al., 2019).

Sickle cell disease poses a considerable public health burden (Katawandja, et al., 2020). It is the most prevalent inherited red blood cell disorder in the United States, affecting around 100,000 individuals. This condition is observed in one out of every 365 births among Black or African American populations and one out of every 16,300 births among Hispanic Americans (Sepulveda, et al., 2023). About 300,000 newly diagnosed sickle cell disease cases are annually born worldwide (Nwabuko, et al., 2022). A systematic study involving sickle cell disease prevalence in 204 countries revealed that, as of 2021, 7.74 million people are living with the disease, marking a 41.4% increase from 2000, resulting in 376,000 mortalities (Thomson, et al., 2023). In the same year, there were 81,100 deaths in children over five years old, ranking sickle cell disease as the 12th highest cause of mortality (Thomson, et al., 2023). The economic burden of managing sickle cell disease is also high. A longitudinal study by Johnson, et al., (2023) revealed that individuals with sickle cell disease covered by private insurance incur around $1.7 million in medical expenses related to the condition throughout their non-elderly life (0-64 years). Additionally, the research demonstrated that individuals faced out-of-pocket expenses amounting to $44,000 throughout their non-elderly lifetimes due to sickle cell disease.

Although sickle cell disease occurs worldwide, it is more prevalent in Africa (Wastnedge, et al., 2018). While the worldwide incidence of sickle cell disease is 112 per 100,000 live births, the prevalence differs significantly across regions. Africa experiences a higher rate of 1125 per 100,000 compared to Europe, which is 43 per 100,000 (Wastnedge, et al., 2018). Sub-Saharan Africa, accounting for about 80% of the global cases of the condition, has the highest prevalence, with Nigeria as the epicenter (Nwabuko, et al., 2022; Adigwe, et al., 2023). The sickle cell trait is found in approximately 23.7% of the population in Nigeria. The incidence of sickle cell disease is around 20 cases per 1000 births, leading to approximately 150,000 babies born annually with sickle cell disease. Without effective and sustainable control strategies, this number is projected to double by the year 2050 (Oluwole, et al., 2020). The fatality rate among children in Nigeria varies from 50% to 80% (Adigwe, et al., 2023). This indicates that the disease is approaching epidemic proportions and requires urgent intervention. The disease burden is exacerbated by inadequate access to comprehensive healthcare in the region.

Contemporary approaches to managing sickle cell disease encompass preventive measures such as prophylactic penicillin and vaccinations to reduce the risk of pneumococcal infections. Additionally, disease-modifying interventions like hydroxyurea are employed, along with blood transfusions for addressing symptomatic acute anemia, stroke management, preoperative optimization, and the consideration of bone marrow transplant (Onimoe & Rotz, 2020). However, these measures must be complemented with appropriate prevention practices to prevent vaso-occlusive crises (Otovwe, et al., 2019). To attain an optimal and stable state of health, children affected by sickle cell disease require optimal family support, comprehension, and caregiving, particularly in ensuring sufficient nutrition and healthcare delivery (Musyoka, et al., 2018). A positive family environment and the implementation of

suitable preventive measures have been demonstrated as favorable indicators for prognosis (Musyoka, et al., 2018). ABO blood group genotypes and demographic characteristics such as gender, age, and marital status can be considered among possible preventive strategies because they have been reported to influence disease prevalence and treatment outcomes in numerous studies, including those by Yahaya, et al., (2022) and Yahaya, et al., (2023). These measures may improve management outcomes when they are factored into sickle cell disease care and management. Unfortunately, in Birnin Kebbi, northwestern Nigeria, there was a dearth of literature on the ABO blood group genotypes and demographic characteristics of sickle cell anemia patients.

OBJECTIVE

The primary objective of the current study was to determine the distribution of ABO blood group genotypes, sickle cell genotypes, and demographic characteristics among sickle cell anemia patients in Birnin Kebbi, Kebbi State, Nigeria.

MATERIAL AND METHOD

Description of the study area

This study was conducted at the Federal Medical Centre in Birnin Kebbi, Nigeria. Birnin Kebbi serves as the capital of Kebbi State in North West Nigeria, situated at latitude 12.4539° N, and longitude 4.1975° E of the equator (Anthony, et al., 2015). It is connected by road to Argungu, Jega, and Bunza. The residents of the town are predominantly of Hausa and Fulani tribes. Birnin Kebbi is located in a tropical region with an average temperature of 32°C and is characterized by seasonal rainfall, typically starting in April and lasting until October, with heavy falls in July and August (Yahaya, et al., 2022). The vegetation in the area consists of sparse trees, such as the neem tree, and a few grasses (Sanni, et al., 2021).

The Federal Medical Centre Birnin Kebbi is the sole federal health institution in Kebbi State. This hospital functions as a referral center for the entire Kebbi State and neighboring states, including Sokoto and Niger States, as well as Niger Republic. This makes the hospital well-suited for the current study.

Data collection

A retrospective and random sampling of sickle cell disease patients who attended the Federal Medical Centre, Birnin Kebbi, between November 2022 and November 2023 was conducted. Relevant medical information about the patients was collected using a standardized checklist. The checklist is comprised of two sections, namely Sections I and II. Section I encompasses socio-demographic variables, including the age, gender, religion, educational status, and ethnicity of each patient. Section II encompasses medical/health information, specifically the ABO blood group genotypes, sickle cell genotypes, and severity of sickle cell anemia. All data were analyzed using the IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, N.Y., USA). This study received approval from the Ethics Committee of Federal University Birnin Kebbi, Kebbi State, Nigeria (Approval number: FUBK025/2023) on 25-09-2023.

Scoring of the severity of the disease

The severity of the disease was evaluated by scoring it based on the frequency of hospital visits or the number of episodes per month. The classification was done according to ABO blood group genotypes. A condition with three visits per month was classified as very severe, twice as severe, once a month as moderately severe, and once every two months as mild.

Sample size determination

The sample size for the study was calculated using equation 1 below (Amoran, et al., 2017).

$$n = \frac{Z2\,pq}{d^2}$$

Where Z is the standard deviation set (1.96), p is the prevalence (0.5), d is the level of precision (0.05), and n (136) is the number of individuals that attended the hospital between November 2022 and November 2023. A total of 99 participants were recruited.

Validation of tool

Statistical validation was systematically performed on each question to assess construct validity. A manual validation process was also implemented for physical records, and a questionnaire was distributed to patients. The questionnaire was meticulously designed to effectively determine individual patients' disease status. These methodological approaches collectively contribute to a robust assessment of validity.

Eligibility criteria

The inclusion criteria comprised eligible medical records of all patients diagnosed with sickle cell anemia between November 2022 to November 2023. Exclusion criteria included individuals outside the specified duration, those with incomplete records, and patients not diagnosed with sickle cell anemia.

RESULT

Demographic data of the patients

Table 1 shows the demographic characteristics of the patients, including age, gender, and religion. The age distribution indicates that the highest number of participants falls within the 1 to 10 age group, comprising 44 individuals (44.4%). Following this, the 11 to 20 age group accounts for 33 participants (33.3%), while the 21 to 30 age group has 17 participants (17.2%). The age groups of 41 to 50 years and 31 to 40 years have 3 (3%) and 2 (2%) patients, respectively. In terms of gender, the study included 48 male participants (48.5%) and 51 female participants (51.5%). Ethnically, the Hausa group constituted the majority, comprising 72 patients (77.7%), followed by the Fulani with nine patients (9.1%), Igbo with eight patients (8.1%), Yoruba with seven patients (7.1%), and Zabarmawa with three patients (3.0%). Examining the religious affiliation of the patients, the majority, 91 individuals (91.90%), identified as Muslims, while eight individuals (8.10%) identified as Christians.

Table 1. Demographic data of the patients.

Variables	Age	Frequency	Percentage %
Age	1-10	44	44.4
	11-20	33	33.3
	21-30	17	17.2
	31-40	2	2.0
	41-50	3	3.0
Total		99	100%
Gender	Male	48	48.5
	Female	51	51.5
Total		99	100%
Religion	Muslim	91	91.9
	Christianity	8	8.1
Total		99	100%
Tribe	Hausa	72	72.7
	Igbo	8	8.1
	Yoruba	9	7.1
	Fulani	7	9.1
	Zabarmawa	3	3.0
Total		99	100%

Figure 1 illustrates the distribution of patients across local government areas (LGAs) in the state. The highest representation was from Birnin Kebbi LGA, accounting for 47%. Jega follows with 8%, Zauro with 7%, Gwandu and Maiyama with 6%, Yawuri with 5%, and Aliero and Kangiwa each recording 4%. Furthermore, Bunza, Kamba, Argungu, and Koko each represented 3% of the total.

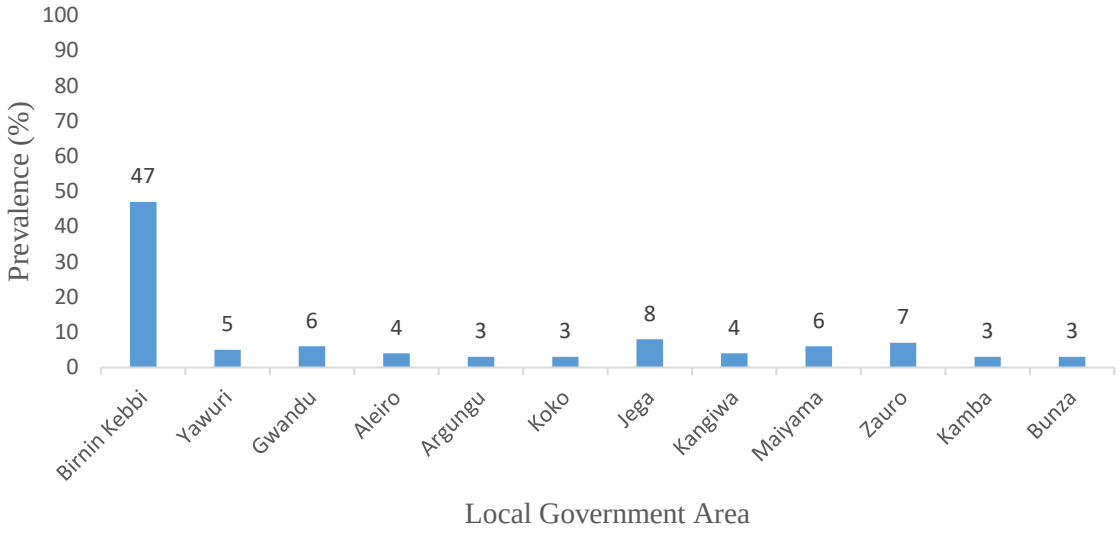

Figure 1. Local government areas (LGAs) of the patients.

ABO blood groups genotypes of the patients

Table 2 shows the ABO blood group genotypes of the patients. Patients with ABO blood group genotypes O^- (33.3%) and A^+ (18.0%) were the most prevalent and least severely affected by the condition, while blood group O^+ (3%) and AB^- (6%) were the least prevalent and most severely affected.

Table 2.Blood group genotypes of the patients.

S/N	Blood group	Frequency	Percentage (%)	Severity
1.	A^+	18	18.2	Mild
2.	A^-	10	10.1	Mild
3.	B^+	6	6.1	Severe
4.	B^-	6	6.1	Severe
5.	AB^+	17	17.2	Mild
6.	AB^-	6	6.1	Severe
7.	O^+	3	3.0	Moderate
8.	O^-	33	33.3	Moderate
Total		99	100%	

Genotypes of sickle cells patients

Figure 2 illustrates the percentages of individuals with sickle cell anemia (hemoglobin SS) and sickle cell carriers (hemoglobin SC) among the patients. Most patients were diagnosed with hemoglobin SS, comprising 92.90%, while those with the carrier status of hemoglobin SC accounted for 7.10%, respectively.

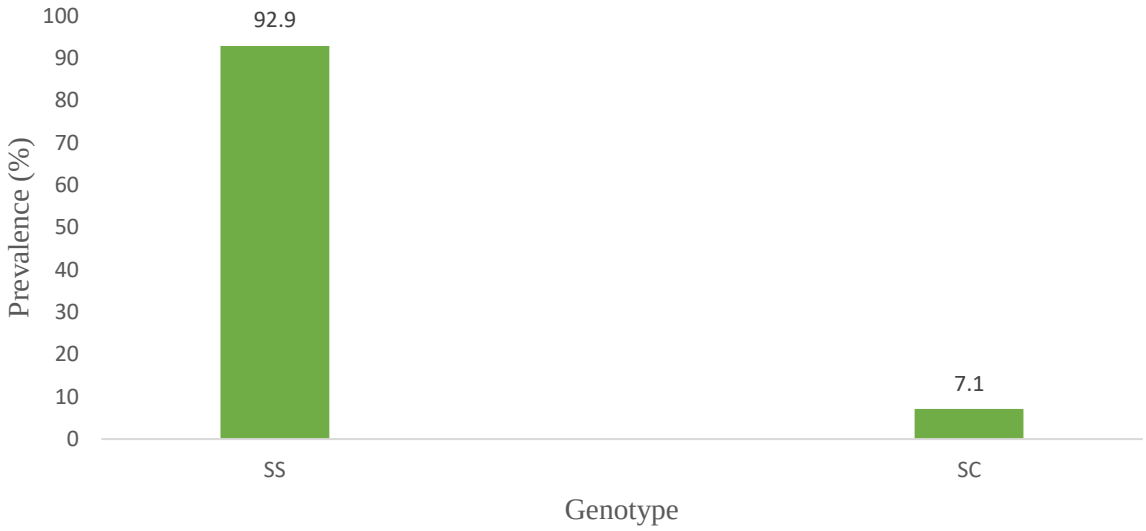

Figure 2. Frequency of SS and SC genotypes among the patients.

DISCUSSION

The primary objective of the current study was to determine the distribution of ABO blood group genotypes, sickle cell variants, and demographic characteristics among sickle cell anemia patients in Birnin Kebbi, Kebbi State, Nigeria. We aimed to provide valuable information for effectively managing sickle cell disease, as these factors have proven useful tools for managing various diseases.

The demographic data obtained revealed that most patients were between 1-10 and 11-20, with a significant proportion between 21-30. This observation was likely due to the early onset of sickle cell disease, a genetic problem that rarely allows affected individuals to reach old age. According to Claeys, et al., (2021), the age of first clinical presentation of sickle cell anemia globally ranges between 2 and 6 years, aligning with the age range observed in our study. Furthermore, in developed nations like the United States, a long-term research by Jiao, et al., (2023) indicated a life expectancy of 52.6 years for sickle cell disease patients at birth, 20 years shorter than healthy individuals. In contrast, in Nigeria, a developing nation with an inadequate healthcare system, the life expectancy at birth was 57.2 for women and 55.1 for men (National Bureau of Statistics, 2023). Based on Jiao, et al., (2023), the life expectancy of sickle cell patients at birth in Nigeria was expected to be less than 40 years, consistent with our study's results. The results of the current study were in line with those of Nwabuko, et al., (2022), who reported the preponderance of pediatric age groups (61%) compared to adult groups (39%) in a study of sickle cell patients carried out in the Federal Medical Center, Umuahia, Abia State, Nigeria. It is also consistent with the findings of Faruk, et al., (2022), who reported a mean age of 6.3 ± 5.1 years for sickle cell patients in a federal hospital in northern Nigeria, of which those under the age of 5 years were 54.1%. Musyoka, et al., (2018) also reported the prevalence of sickle cell disease among children between 1 and 10 years in Tanzania. The demographic information further reveals no significant gender disparity in the disease occurrence as males accounted for 48.5% and females (51.5%), which are close figures. Similar gender proportion closeness was also reported by Adigwe, et al., (2023) among sickle cell anemia patients in Abuja, Nigeria; however, male participants were in the majority, accounting for 52%. Contrarily, Nwabuko, et al., (2022) reported the preponderance of females (56.6%, n=64) compared to males (43.4%, n=49) among sickle cell patients in Umuhahia, Abia State, Nigeria. Faruk, et al., (2022) also reported more males, 286 (62%), than females, 174 (38%), in a survey of sickle cell disease carried out at a federal medical facility in northern Nigeria. The demographic information further revealed that the participants were dominated by the Hausa ethnic group and Muslims, which reflect the dominant ethnic group and the religion of the people of the study area.

The distribution of ABO blood group genotypes among the patients revealed that individuals with O^- blood type constituted the majority, followed by A^+, AB^+, and A^-, with O^+ being the least prevalent. A study conducted by Alagwu, et al., (2016) in Asaba, Delta State, Nigeria, found a similar dominance of

the O blood group. However, the authors did not specify the proportion of negative or positive types. Nwabuko, et al., (2022) also reported a prevalence of the O blood group among sickle cell patients in Umuahia, followed by A and B blood groups, respectively. Similarly, in a study by Nwabuko, (2017) in Port Harcourt, Nigeria, the O blood group was predominant, followed by A and B blood groups, while the AB blood group was absent. A study by Sacomboio, et al., (2020) in Angola revealed that blood group O was predominant, followed by A, and the combination of these two accounted for well over half of the participants, mirroring our study's findings. Furthermore, our results indicated that the condition was less severe among individuals with the O blood group than those with non-O blood groups. Severity was also lower among individuals in the A blood group, although not as pronounced as in the O blood group. The reduced severity of the condition among O and A blood groups suggests that the relatively high prevalence of the disease in these groups may indicate their protective nature. Amsalu & Daniel, (2019) proposed that the dominance of group O blood could indicate resistance or protection from diseases, representing an evolutionary success. Studies by Ahmed, et al., (2014) and Akpan & Asuquo, (2022) demonstrated that sickle cell patients with non-O blood groups had higher frequencies and risks of vaso-occlusive crises (VOC), attributed to higher levels of von Willebrand factor-making non-O blood groups a risk factor for frequent VOC and an adverse prognostic index in sickle cell disease. Additionally, a study by Amodu, et al., (2012) in southwest Nigeria found that O and A blood groups were predominant among sickle cell patients, with a decreased risk of malaria, unlike the less dominant B blood group, which showed an increased risk. Therefore, the lower severity observed in the O blood group could be due to its protective effect against diseases associated with sickle cell disease, such as malaria. However, some scientists suggest that group O blood is generally and universally common due to being the ancestral ABO blood group; A and B alleles appeared in the last 20,000 years and have not spread as much as O in the population Yahaya, et al., (2021). Nonetheless, this assertion still indirectly points to the evolutionary success of the O blood group, embodying the concept of 'survival of the fittest.'

Regarding the genotypes of the sickle cell patients, two variants, hemoglobin SS and SC, were identified, with SS being the predominant genotype. Consistent with our current study, Nwabuko, et al., (2022) reported a prevalence of SS over SC in Umuahia, and Kingsley, et al., (2019) also found SS to be more dominant than SC in Calabar, Nigeria. However, our study revealed a higher proportion of SC than other studies, with 7.1% SC reported, as opposed to the 0.14% reported by Kingsley, et al. (2019). Additionally, Nwabuko, et al., (2022) recorded 98.55% for SS compared to 1.45% for SC. According to Nwabuko, et al., (2022), studies indicate that while hemoglobin SS is evenly distributed across all geopolitical zones of Nigeria, hemoglobin SC is less prevalent and more concentrated in western Nigeria, where it could account for about 3-4%, but is least prevalent in the east of the Niger delta river. It can then be hypothesized that the comparatively higher prevalence of SC observed in our study suggests the influence of a modifying factor, potentially favoring the fitness and inheritance of the SC variant or indicating an increased mutation from SS to SC. However, further studies are needed to validate these claims.

Strength and limitations

The strength of this study lies in its ability to clearly define the distribution of demographic information and ABO blood group genotypes among sickle cell anemia patients in the study area. However, the study is constrained by a small sample size, attributed to restrictions imposed by hospital management.

CONCLUSION

Demographic characteristics affected the distribution of sickle cell anemia in the city, with the disease showing a higher prevalence among children aged 1 to 10 and those aged 11 to 20. Furthermore, the disease affected the Hausa ethnic group and Muslims more than other ethnic groups. However, there was no gender disparity in the occurrence of the disease. ABO blood group genotypes also influenced susceptibility to the disease: individuals with blood genotype O^-, accounting for 33.3%, and A^+, accounting for 18.0%, were the most prevalent and least severely affected by the condition, while blood groups O^+ (3%) and AB^- (6%) were the least prevalent and most severely affected. Moreover, HbSS and HbSC were the dominant sickle cell anemia variants identified among the participants, with HbSS

(92.9%) being the most prevalent. These findings show that attention should focus equally on males and females. Particular attention should be directed towards individuals with O^+ and AB^- blood groups in the city, as they exhibit the most severe form of the disease. Considering the comparatively significant proportion of HbSC in the town, which is less severe, advocating for marriages between individuals with HbSS and HbSC is advised to decrease the prevalence of HbSS while increasing HbSC. This study suggests the need for similar research with a larger sample size and the inclusion of more hospitals to obtain a more comprehensive understanding of the prevalence and characteristics of sickle cell disease in the city.

Acknowledgment
The authors would like to thank the board of directors and staff of the Federal Medical Center, Birnin Kebbi, Nigeria, for their assistance in conducting this study.

Conflict of Interest
All authors have no conflict of interest.

Ethic Consideration
This study received approval from the Ethics Committee of Federal University Birnin Kebbi, Kebbi State, Nigeria (Approval number: FUBK025/2023) on 25-09-2023. The guidelines for researching humans, as outlined by the Committee, were strictly adhered to. Additionally, informed consent was obtained from the hospital.

Funding Disclosure
None

Author Contribution
TOY contributed to the conception and design, drafting of the article, critical revision of the article for important intellectual content, and final approval. AKA and AS contributed to the analysis and interpretation of the data and the collection and assembly of data. ABI contributed to statistical expertise. MKA and AZF contributed to the provision of study materials for patients.

REFERENCES

Abboud, M. R. 2020. Standard management of sickle cell disease complications. Hematology/Oncology and Stem Cell Therapy, 13(2): 85–90. doi: 10.1016/j.hemonc.2019.12.007.

Adigwe, O. P., Onoja, S., Onavbavba, G. 2023. A critical review of sickle cell disease burden and challenges in Sub-Saharan Africa. Journal of Blood Medicine, 14: 367–376. doi: 10.2147/JBM.S406196.

Ahmed, S., Kagu, M., Ibrahim, U. 2014. Correlation between ABO blood group and vaso-occlusive crisis among adult patients with sickle cell anemia in northern Nigeria. The Egyptian Journal of Haematology, 39(4): 227. doi: 10.4103/1110-1067.153964.

Akpan, I., Asuquo, I. E. 2022. Assessing the relationship between plasma von Willebrand factor antigen levels, ABO and Rh (D) blood groups and risk of sickle cell anaemia vaso – occlusive crisis. Saudi Journal of Medicine, 7(8): 428–434. doi: 10.36348/sjm.2022.v07i08.006.

Alagwu, E. A., Akukwu, D., Ngwu, E. E., et al. 2016. ABO/rhesus blood group and correlation with sickle cell disease and type-II diabetes mellitus in South East and South-South of Nigeria. Pharmaceutical and Biosciences Journal: 78–82. doi: 10.20510/ukjpb/4/i5/134669.

Amodu, O. K., et al. 2012. Association of the sickle cell trait and the ABO blood group with clinical severity of malaria in southwest Nigeria. Acta Tropica, 123(2): 72–77. doi: 10.1016/j.actatropica.2012.03.013.

Amoran, O. E., Jimoh, A. B., Ojo, O., et al. 2017. Prevention practices influencing frequency of occurrence of vaso-occlusive crisis among sickle cell patients in Abeokuta South Local Government Area of Ogun State, Nigeria. BMC Hematology, 17(1): 6. doi: 10.1186/s12878-017-0077-9.

Amsalu, W. F., Daniel, G. D. 2019. Distribution of ABO and Rh (D) blood groups among students attending secondary and preparatory schools in Bote town, Oromia national regional state, Ethiopia.

International Journal of Science and Technology Education Research, 10(1): 1–8. doi: 10.5897/IJSTER2019.0448.

Anthony, A., Dabara, D. I., Oni, S. S., et al. 2015. Urbanization dynamics and land use pattern in Birnin Kebbi, Nigeria. Conference of the International Journal of Arts & Sciences, 8(1), pp. 41–52. Available at: https://papers.ssrn.com/sol3/papers.cfm?abstract_id=2784498.

Claeys, A., van Steijn, S., van Kesteren, L., et al. 2021. Varied age of first presentation of sickle cell disease: Case presentations and review. Case Reports in Medicine. Edited by F. J. Walther, 2021: 1–7. doi: 10.1155/2021/8895020.

Faruk, J., Adebiyi, M., Ahmad, H. 2022. Clinico-demographic characteristics, morbidity and mortality patterns of sickle cell disease in a tertiary institution. Sahel Medical Journal, 25(2): 52. doi: 10.4103/smj.smj_78_20.

Hussaini, M. A., Durbunde, A. A., Jobbi, Y. D., et al. 2019. Assessment of experience, perception and attitude towards premarital sickle cell disease screening among students attending Federal College of Education, Kano, Nigeria. International Journal of Research and Reports in Hematology, 2(1): 15–26. Available at: https://journalijr2h.com/index.php/IJR2H/article/view/8.

IBM Corp. 2012. IBM SPSS statistics for Windows, version 21.0. Armonk, NY: IBM Corp. Available at: https://translate.google.com/translate?u=https://www.ibm.com/support/pages/downloading-ibm-spss-statistics-21&hl=id&sl=en&tl=id&client=srp&prev=search

Jiao, B., Johnson, K. M., Ramsey, S. D., et al. 2023. Long-term survival with sickle cell disease: A nationwide cohort study of Medicare and Medicaid beneficiaries. Blood Advances, 7(13): 3276–3283. doi: 10.1182/bloodadvances.2022009202.

Johnson, K. M., Jiao, B., Ramsey, S. D., et al. 2023. Lifetime medical costs attributable to sickle cell disease among nonelderly individuals with commercial insurance. Blood Advances, 7(3): 365–374. doi: 10.1182/bloodadvances.2021006281.

Katawandja, A. L., Kingwengwe, A. A., Mwamba, E. S. 2020. Knowledge and practices of health providers on the diagnosis and biological monitoring of sickle cell disease in the City of Kindu, in the East of the Democratic Republic of Congo. OALib, 7(9): 1–11. doi: 10.4236/oalib.1106757.

Kingsley, A., Enang, O., Essien, O., et al. 2019. Prevalence of sickle cell disease and other haemoglobin variants in Calabar, Cross River State, Nigeria. Annual Research & Review in Biology: 1–6. doi: 10.9734/arrb/2019/v33i530132.

Lu, D. C. Y., Wadud, R., Hannemann, A., et al. 2021. Pathophysiological relevance of renal medullary conditions on the behaviour of red cells from patients with sickle cell anaemia. Frontiers in Physiology, 12. doi: 10.3389/fphys.2021.653545.

Mbiya, B. M., Disashi, G. T., Gulbis, B. 2020. Sickle cell disease in the Democratic Republic of Congo: Assessing physicians' knowledge and practices. Tropical Medicine and Infectious Disease, 5(3): 127. doi: 10.3390/tropicalmed5030127.

Musyoka, A. M., Zebedayo, K., Mmbaga, B. T. 2018. Prevalence of sickle cell disease among anaemic children attending Mbeya Referral Hospital in Southern Tanzania. The East African health research journal, 2(2): 142–146. doi: 10.24248/EAHRJ-D-18-00015.

National Bureau of Statistics. 2023. Statistical report on women and men in Nigeria, National Bureau of Statistics. Available at: https://www.nigerianstat.gov.ng/pdfuploads/2021_Statistical_Report_On_Women_and_Men.pdf.

Ngwengi, N. Y., Nde Fon, P., Fon Mbanya, D. 2020. Distribution of haemoglobin genotypes, knowledge, attitude and practices towards sickle cell disease among unmarried youths in the Buea Health District, Cameroon. Pan African Medical Journal, 37. doi: 10.11604/pamj.2020.37.109.17864.

Nwabuko, O. C. 2017. Assessment of ABO-rhesus blood groups and hemoglobin concentrations of sickle cell disease pregnant woman at Booking in Nigeria. Hematology & Transfusion International Journal, 5(2). doi: 10.15406/htij.2017.05.00113.

Nwabuko, O. C., Onwuchekwa, U., Iheji, O. 2022. An overview of sickle cell disease from the socio-demographic triangle - a Nigerian single-institution retrospective study. Pan African Medical Journal, 41. doi: 10.11604/pamj.2022.41.161.27117.

Oluwole, E. O., Adeyemo, T. A., Osanyin, G. E., et al. 2020. Feasibility and acceptability of early infant screening for sickle cell disease in Lagos, Nigeria—A pilot study. PLOS ONE. Edited by A. Wonkam, 15(12): e0242861. doi: 10.1371/journal.pone.0242861.

Onimoe, G., Rotz, S. 2020. Sickle cell disease: A primary care update. Cleveland Clinic Journal of Medicine, 87(1): 19–27. doi: 10.3949/ccjm.87a.18051.

Onoja, S. O., Eluke, B. C., Dangana, A., et al. 2021. Evaluation of von Willebrand factor and other coagulation homeostasis profile of patients with sickle cell anemia attending a Tertiary Hospital in Enugu, Nigeria. Medical Journal of Zambia, 47(4): 269–275. doi: 10.55320/mjz.47.4.715.

Otovwe, A., Sunday, U. I., Rumi, O. B., et al. 2019. Knowledge and attitude of premarital genotype screening among women of child-bearing age in Kumo-Akko Local Government Area of Gombe State Nigeria. Open Journal of Public Health, 1(2): 1–4. Available at: https://www.remedypublications.com/open-journal-of-public-health-abstract.php?aid=5591.

Sacomboio, E. N. M., Sebastião, D. T., Sacomboio, F. de J. C. F. 2020. Sickle cell trait and blood groups (ABO and Rh) in Angolans submitted to hemoglobin electrophoresis. Hematology and Oncology: Current Research, 2(1), pp. 1–7. Available at: https://meddocsonline.org/hematology-and-oncology-current-research/sickle-cell-trait-and-blood-groups-ABO-and-Rh-in-angolans-submitted-to-hemoglobin-electrophoresis.pdf.

Sanni, U. A., Lawal, T. O., Musa, T. L., et al. 2021. Prevalence and outcome of snake bites among children admitted in the Emergency Pediatric Unit, Federal Medical Centre, Birnin Kebbi, Nigeria. Cureus, 13(8). doi: 10.7759/cureus.17413.

Sepulveda, J. M. G., Yang, J. C., Reed, S. D., et al. 2023. Preferences for potential benefits and risks for gene therapy in the treatment of sickle cell disease. Blood Advances, 7(23): 7371–7381. doi: 10.1182/bloodadvances.2023009680.

Thomson, A. M., McHugh, T. A., Oron, A. P., et al. 2023. Global, regional, and national prevalence and mortality burden of sickle cell disease, 2000–2021: A systematic analysis from the Global Burden of Disease Study 2021. The Lancet Haematology, 10(8): e585–e599. doi: 10.1016/S2352-3026(23)00118-7.

Wastnedge, E., Waters, D., Patel, S., et al. 2018. The global burden of sickle cell disease in children under five years of age: A systematic review and meta-analysis. Journal of Global Health, 8(2). doi: 10.7189/jogh.08.021103.

Yahaya, T., Abdullahi, H., Muhammad, Z., et al. 2023. ABO blood group genotypes and demographic traits in susceptibility to type 1 diabetes mellitus in Lagos, Southwest, Nigeria. Nigerian Journal of Physiological Sciences, 37(2): 199–205. doi: 10.54548/njps.v37i2.6.

Yahaya, T. O., Oladele, E. O., Mshelia, M. B., et al. 2021. Influence of ABO blood groups and demographic characteristics on the prevalence of type 2 diabetes in Lagos, southwest Nigeria. Bulletin of the National Research Centre, 45(1): 144. doi: 10.1186/s42269-021-00603-0.

Yahaya, Ta., Oladele, E., Ahmed, H., et al. 2022. Distribution of ABO blood groups and selected diseases among students of Federal University Birnin Kebbi, Nigeria. Suranaree Journal of Science & Technology, 29(1): 1–8. Available at: https://www.researchgate.net/publication/358967753_DISTRIBUTION_OF_ABO_BLOOD_GROUPS_AND_SELE CTED_DISEASES_AMONG_STUDENTS_OF_FEDERAL_UNIVERSITY_BIRNIN_KEBBI_NIGERIA.

ORIGINAL RESEARCH REPORT

Does the Nias Tribe's Head Circumference Correlate with Body Height?

Windy Juwita Lumbanraja[1], Saharnauli Janna Verawaty Simorangkir[2*], Joseph Partogi Sibarani[3]

[1]Bachelor of Medicine Education Program, Faculty of Medicine, HKBP Nommensen University, Medan, Indonesia.
[2]Department of Anatomy, Faculty of Medicine, HKBP Nommensen University, Medan, Indonesia.
[3]Department of Internal Medicine, Faculty of Medicine, HKBP Nommensen University, Medan, Indonesia.

Article Info

Article history:
Received 26-02-2024
Revised 03-06-2024
Accepted 10-06-2024
Published 01-07-2024

Keywords:
Height
Height circumference
Forensic identification
Anthropometry
Public health

*__Corresponding author__:
Saharnauli Verawaty Simorangkir,
saharnauli@uhn.ac.id

ABSTRACT

Background: Anthropometric measures are one of the protocols frequently employed to identify deceased bodies. Height, age, and gender are a few anthropometric traits that might be utilized to identify the victim from the corpse. If body components are absent, head circumference can be used to determine body height. **Objective**: The goal of the study was to ascertain whether there is a correlation between head circumference and body height by gender and to create formulas for estimating weight based on head circumference in the Nias tribe at the University of HKBP Nommensen Medan, Indonesia. **Material and Method**: The study was conducted from September to October 2023 at the University of HKBP Nommensen using an observational cross-sectional design. Of the 100 students who met the inclusion criteria, 50 were men, and the remaining 50 were women. The study's dependent variable was micro-toise-measured height. The head circumference, expressed in meters of tape, served as the study variable. **Result**: *S*tatistical analysis of the study results from a significant correlation between the head circumference and the height ($r = 0.655$, $p<0.01$). The linear regression equation obtained was $y = 3200 x - 2.672$ in men and $y = 10970 + 2.728$ times in women, where y was height and x was head circumference. **Conclusion**: A substantial height-head circumference association was found among the Nias tribe at the University of HKBP Nommensen Medan, Indonesia. The identification method of the forensic field may benefit from this research.

How to cite:
Lumbanraja, W.J., Simorangkir, S.V., Sibarani, J.P. 2024 Does the Nias Tribe's Head Circumference Correlate with Body Height?. Majalah Biomorfologi-Biomorphology Journal, 34(2): 93-101.

Majalah Biomorfologi (Biomorphology Journal) p.ISSN:0215-8833, e.ISSN: 2716-0920
doi: 10.20473/mbiom.v34i2.2024.93-101

Highlights

1. A significant correlation was found between head circumference and height among Nias tribe students.
2. This study was conducted to obtain formulations for weight determination based on headbands in the Nias tribe.

BACKGROUND

Identification is an effort to support an investigator in fulfilling the visum et repertum requirement for a person's identification. As such, it can be helpful evidence in a criminal prosecution regarding a person's health, life, or body (Poluan, et al., 2016). Determining the identity of both the living and the dead, including those with the means to change their identity, such as murderers, criminals, rapists, parents who have switched babies, people undergoing cosmetic surgery to alter their appearance, and those suffering from memory loss requires identification in forensic medicine (Romdhon, 2015). In the event of a mass disaster, forensic identification can play a crucial role in assuring the victim's family of their identity and offering them psychological comfort (Saputri & Junitha, 2023).

In Indonesia, much information is shared regarding natural disasters that have caused significant damage, such as landslides, road accidents, tsunamis, and earthquakes. The Indonesian National Disaster Management Authority (BNPB) has published data indicating that there has been a rise in disasters in Indonesia so far in 2023, which has contributed to an increase in event charts over time (National Disaster Management Authority, 2022). The 9.3 magnitude retail scale tsunami that struck Aceh on December 26, 2004, is one of the natural disasters. More than 250,000 people lost their lives in an earthquake and massive tsunami that followed earlier. This incident is Indonesia's record for the highest tsunami ever recorded (National Disaster Management Authority, 2023). Another earthquake occurred in Yogyakarta 17 years ago, on May 27, 2006, with a force of 5.9 retail scale, which took thousands of lives (Putri & Kurniawan, 2022). Among these incidents, a common issue is difficulty in recognizing the victims' bodies when the limb damage is so severe that only a few body pieces remain (Imran, 2015).

Anthropometrics means measuring the human body, derived from the Latin word anthropos, which means human, and metron, which means measuring (Tur & Bibiloni, 2019). According to Sanders & McCormick, (1976) anthropometrics is the study of body dimensions that are important for the design of objects that other people use. Measurements of the body skeleton proportion that have been measured are made in an anthropometric order. Gender, race, origin, height, and other factors are also considered in the forensic science of human identity. The application of techniques for legitimate goals and scientific procedures both make use of forensic science. Some have suggested that because certain ethnicities differ in diet and climate, one forensic science of race's characteristics calls for its height measurement. Thus, additional research from every area must be taken into consideration. (Reddy, et al., 2018).

Anthropology does play a vital role in identifying humans. A critical parameter for identifying humans both in life and death is posture. One method used in estimating the body height of partial remains is, therefore, very useful in forensic science because of such disasters as suicide, tsunamis, earthquakes, wildfires, airplane accidents, and so on, is a technique used to approximate height by measuring specific body regions. The most visible posture is based on an excellent upright height and a definite biological link, as well as on each body part, such as the face, the head, the extremes, and the nose. Height estimates do not pose a problem if the body of the deceased is found intact. Anthropometric measures can help identify the body of the victim with a known identity or even an unknown identity and thus make it easier for investigators to search for the victim's identity (Prenetha & Babu, 2022).

The size of the spine, limb bones, neck, and skull are among the anthropological criteria for determining body height during identification. According to Shah, et al., (2017), research in India has demonstrated that head circumference can be used to estimate a person's height. A research by Larasati, et al., (2018) showed that head circumference has a robust correlation with body height in men of the Mongoloid race in Pandean, Surabaya. In another study by Bharti, et al., (2019), findings from a study on the Haryanvi people in the Panchkula area indicated a significant relationship between head circumference and body height. Research by Eboh & Ohaju-Obodo, (2019) defined a regression model for height estimates based on specific population characteristics and gender traits in particular populations and genders. According to the study's findings, a straightforward regression model incorporating medicolegal factors can assess a person's height based on their head circumference.

Indonesia is the most significant island nation in the world, with 17,508 islands. There are 360 different ethnic groups, each with unique cultural traditions and climates. Tropical conditions support a diverse range of terrestrial and marine life (Illahi, 2021). The statistical central body census collected

data showing more than 300 ethnicities and 1,340 Indonesian tribal groups as of 2010 (Graha, 2023). The province of North Sumatra is home to several different tribes, including the Nias, Javanese, Malay, and Batak. According to some estimates, there were 41.9% of the Batak tribe, 32.6% of the Javanese, and 6.3% of the Nias tribe. Based on data from the Civil Registry Service Office (*Dukcapil*), which registered around 890 thousand tribal individuals in North Sumatra in December 2021, the Nias is ranked as the third largest tribe in North Sumatra (Jayani, 2021). However, for the Nias tribe, specific anthropometric information is still scarce for forensic and identification purposes. This is urgent, given Indonesia's numerous natural disasters and criminal prosecutions. Thus, we aimed to investigate the correlation between body height and head circumference among the Nias tribe of North Sumatra.

OBJECTIVE

The study aimed to determine whether head circumference and gender-specific height were correlated and to develop formulas for determining headband-based height in Nias students at the University of HKBP Nommensen Medan.

MATERIAL AND METHOD

This study method was descriptive observational research using a sectional view design where the data collection occurs only once in a given moment. The study was conducted from September to October 2023 at the University of HKBP Nommensen using an observational cross-sectional design. Of the 100 students who met the inclusion criteria, 50 were men, and the remaining 50 were women. The study's inclusion requirements included being a current student at HKBP Nommensen University between the ages of 20 and 30, belonging to the Nias ethnic group, not having married into another ethnic group for at least two generations, and being willing to sign an informed consent form. In the meantime, having congenital deformities affecting the skull, trauma or dislocation affecting body height, having undergone or are undergoing a height-affecting dislocation, fracture, trauma, or injury; having previously undergone height-affecting surgical therapy; having a history of growth hormone imbalances or birth defects (such as dwarfism and acromegaly); and having a history of spinal abnormalities (lordosis, kyphosis, scoliosis) were the exclusion criteria for this investigation. The Health Research Ethics Community, Faculty of Medicine HKBP Nommensen University approved the study, with approval number 535/KEPK/FK/IX/2023 on 12 -09-2023.

In this study, head circumference was the dependent variable, and the body height was the independent variable. A microtoise was used to measure people's heights. The individual stands straight, with their arms hanging at their sides and their head aligned with the Frankfort Horizontal Plane (FHP). With the feet together and the body upright, measurements were taken from the vertex, or highest point on the head, to the heel. The measurer lines up the eye parallel to the height measuring rod and reads the measurement by glancing at the number printed on the microtoise. Following the decimal point, results were reported in centimetres to one digit. The measurement results were recorded in the questionnaire's height column under the respondent's identification information. Head circumference measurements were made while the participant was seated in the FHP plane at the level slightly above the glabella, or the midpoint between the brow ridges, to the opisthocranion perpendicular to the midsagittal plane. Pressing the tape on the hair requires hard pulling. The tape should not be allowed to slide past the head by using the middle finger on the side of the head. The ears were prevented from being inserted. Hairpins, clips, or similar objects would not be poked into the hair during the measurement. The measurement findings were recorded in the questionnaire's head circumference field. Both variables underwent three separate measurements, each performed by a trained individual. The average values were the measurement findings that were utilized. Every day, at the same time, between 08.00-09.00 am, measurements were taken until the necessary sample size was obtained.

The iPhone "Scoliometer" version 6.1 was used for scoliosis evaluation to rule out exclusion criteria. An examination was conducted to determine the Trunk Rotation Angle (ATR). The respondent must remove all clothing from the upper body during the scoliometer examination to fully view the ribs and spine. Wearing a sleeveless T-shirt and a head knot was necessary for ladies, but men can go bare-chested.

To evaluate the respondents, they had to bend forward until their shoulders were parallel to their hips. The respondents were instructed to stand with their knees straight, their feet parallel, and their hands hanging loose (Figure 1). The replies were then leans forward, feet together, knees straight, and hands either dangling freely (Safitri, et al., 2020). The cellphone's long side must be positioned at the top of the spine, above the spinous process, without being pressed, for the scoliometer program to work (Shaw, et al., 2012). Next, the curve degree numbers displayed on the software were read. Regarding early detection, values below 5° were deemed negligible and had little bearing on the outcomes of height measures (Nabila, 2020).

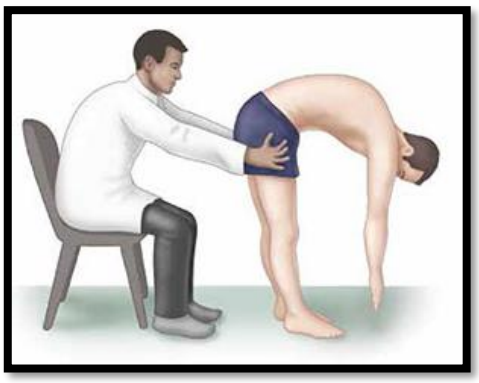

Figure 1. Forward bending position.

Kyphosis and lordosis assessments were carried out with the "Goniometer" iPhone app version 4.0.1. The phone's short side was positioned over the anatomical landmarks (T1–T3, T12, and L1) indicated on the spine for the goniometer software to function. The protractor was first set to 0°, and the phone was placed on the spinous processes of T1–T3. After that, the phone was positioned on the spinous processes of T12 and L1, and the angle shown on the screen was noted as the Thoracic Kyphosis Angle (TKA). The mobile's short side was positioned above the spine's anatomical markers (L1, L5) to measure the lordosis angle. The protractor was set to 0°, and the phone was positioned in L1's spinous process. Next, the cell phone was placed on the L5 spinous process, and the angle value displayed on the screen was recorded as Lumbar Lordosis Angle (LLA) (Elpeze, et al., 2023).

Data distribution was presented in a descriptive form with deviational standards and an average of normal data or a maximum distribution and value for the spread of abnormal data. Because the sample size was greater than 50, the data was examined using the Kolmogorov-Smirnov normality test on the entire data set. Next, a linearity test was performed to ascertain whether the data were appropriate for testing via a correlation test. The correlation test should be performed if there is a linear relationship between the two data sets; otherwise, it should not be employed. Positive linearity implies that as one variable's value rises, the value of another variable also increases. In contrast, negative linearity shows that when one variable's value rises, the value of the other variable falls. When testing for correlation, linear data will be put through the Pearson correlation test if the distribution of the two data is normal and the Spearman correlation test if not. In the p-value correlation test, a value of less than 0.05 indicates a significant correlation between the two variables under investigation. In contrast, a value greater than 0.05 indicates no considerable association (Dahlan, 2021). Scientists worldwide have employed the regression approach and the multiplication method extensively for this kind of computation, and they have all concluded that regression analysis offers the best estimates for stature reconstruction (Krishan, et al., 2010). Thus, linear regression analysis was applied to create a regression equation that may link this study's dependent and independent variables. Statistical analysis used IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA) and Microsoft Office Excel 2016.

RESULT

A sampling method was used to acquire samples from up to 100 individuals. According to Table 1, the distribution of sample characteristics based on the 21-year-old span was 67 people (67.0%), 23 people (23.0%) who were 22 years old, and 10 people (10.1%) who were 23 years old. Fifty males (50.0%) and fifty women (50.0%) comprised the gender distribution.

Table 1. Sample characteristics.

Variable	Category	Total	Percentage
Age	21	67	67.0%
	22	23	23.0%
	23	10	10.0%
Sex	Male	50	50.0%
	Female	50	50.0%

Table 2 shows the values of the two variables. The male sex's average height was 167.4 cm (SD=5.16), whereas the female sex's average height was 155.4 cm (SD=5.18). Male sex-related head circumference values were 54.15 cm (SD=1.46) and 52.97 cm (SD=1.87) for women. Descriptive statistics showed that the two variables were more significant in men than women. Table 2 reveals that p=0.000 indicates a significant association between the height of both sexes and head circumference. The head circumference and the height of the two sexes had a high positive link, as indicated by Pearson's Pearson correlation values of 0.908 and 0.986.

Table 2. Variable description based on gender.

Variable	Female			Male		
	Mean	SD	r (p-value)	Mean	SD	r (p-value)
Height	167.4	5.16	0.908 (0.000)	155.4	5.18	0.986 (0.000)
Head circumference	54.15	1.46		52.97	1.87	

Table 3 shows that regression analysis from a simple, linear analysis obtained the value of an independent variable constant of -2.609 and a 3.200 regression coefficient, with a value of p=0.000 (p< 0.05).

Table 3. Simple linear regression analysis.

Variable		Constant	Coefficient	Standard error of the estimate	p
Head circumference and height	Male	-2.672	3.200	2.1872	<0.001
	Female	10.970	2.728	0.8689	<0.001

Simple linear regression equations based on sex as follows:
1. On the male sample :
Y = (-2.672) + 3.200 X
Y = (-2.672) + 3.200 (head circumference)
Y = 3200 (head circumference) – 2.672

2. On the female sample:
Y = 10.970 + 2.728 X
Y = 10.970 + 2.728 (head circumference)

Description:
X = Head circumference
Y = Body height

DISCUSSION

The HKBP University Nommensen Medan is home to a diverse range of tribes because its students are from all parts of Indonesia. Approximately one hundred participants who satisfied the inclusion and exclusion criteria were included in the study. The measurements showed that the female form measured 155.4 cm, and the male figure was 167.4 cm on average. This was comparable to past studies on the Mongoloid race by Afandi, et al., (2021) when it was discovered that women had a flatbed measuring 156.2 cm and men had a flatbed measuring 167.3 cm. However, a notable finding from a study by Prenetha & Babu, (2022) at Saveetha Dental College on first-year students between the ages of 18 and 20 years old was that women's height was 158.62 cm and men's was 173.54 cm. Eboh & Ohaju-Obodo, (2019) study at six universities in southern Nigeria, students with Bekware, Esan, Kalabari, Ogbia, and Urhobo who were 18 to 30 years old obtained height in male 151 cm and female150 cm. This could be the case due to various factors influencing height growth, including nutrition, race, and environment.

The head circumference of the male was 54.15 cm larger than the woman's, measuring 52.97 cm, according to the findings of the univariant test of head circumference. This contrasts previous research on young Gujarati people between 18 and 22, which discovered that the female's head circumference measured 50.5 cm and the male's measured 55.6 cm (Bharti, et al., 2019). Additional research conducted in southern Nigeria yielded similar head-girl-line results of men measuring 52.1 cm and women measuring 54.1 cm (Eboh & Ohaju-Obodo, 2019).

A Pearson test for the line-ring correlation between the height of the body and the head circumference was the statistical outcome of this investigation. The head circumference and height had a high and substantial link, with a coefficient correlation of r =0.908 and a significant p-value of 0.000 for females and r=0.986 and a significant p-value of 0.000 for males. These findings in both the female and male groups demonstrated a very high association between head circumference and body height. This study correlated with a study conducted on the Mongoloid by Afandi, et al., (2021), which found a strong and substantial association between height and head circumference among the students of the University Medical School ($p<0.05$). According to a study by Febriawan, (2015), also on a Mongoloid race, there was a strong and significant correlation of $r = 0.61$ ($p<0.05$). The research conducted by Mansur et al. at Kathmandu University School of Medical Sciences in Dhulikhel, Nepal, from November 2011 to October 2012 revealed a strong correlation between height and head circumference. The correlation coefficients were 0.443 ($p < 0.01$) for males, 0.302 ($p < 0.01$) for females, and 0.398 ($p < 0.01$) for both genders combined. The regression equation for the relationship between height and head circumference was determined to be $Y = 1.734X + 70.36$ ($R2=0.196$) for males, $Y = 0.916X + 106.8$ ($R2= 0.091$) for females, and $Y = 1.648$ for the overall population (Mansur, et al., 2014). A study by Eboh & Ohaju-Obodo, (2019), conducted at six universities in southern Nigeria and involving students aged 18 to 30, has yielded a different conclusion from this study. Specifically, their association was modest, with $r=0.48$ ($p<0.05$). Additional research that supported Eboh & Ohaju-Obodo, (2019) findings was carried out at Saveetha Dental College on first-year students between the ages of 18 and 20, consisting of 35 males and 35 girls. The results showed a small relationship with $r = 0.45$ ($p=0.05$) (Prenetha & Babu, 2022).

Based on the information above, head circumference is among the best parameters discovered to have a strong and substantial link with an individual's height. Thus, in medical-legal investigations and anthropometry, if one of the two measurements, i.e., the person's height or head circumference, is known, the other can be calculated. A fundamental linear regression analysis was conducted on this study, yielding a significance value of 0.000. A value of <0.05 was considered significant.

Strength and limitations

The benefit of this research is that it provides a formula for calculating an individual's height using their head circumference measurements. Additionally, the study was conducted particularly for the Nias tribe, one of the ethnic groups that predominately resides in Medan. The research has a problem in that it does not categorize the Nias tribe according to their geographical areas of residency, which significantly impacts how the human body is shaped anatomically.

CONCLUSION

There was a significant correlation between head circumference and height. This research can help in the identification process in the forensic field.

Acknowledgment

We thank Universitas HKBP Nommensen Medan for facilitating this research.

Conflict of Interest

According to the author(s), no conflict of interest exists.

Ethic Consideration

The Health Research Ethics Community, Faculty of Medicine HKBP Nommensen University approved the study, with approval number 535/KEPK/FK/IX/2023 on 12 -09-2023.

Funding Disclosure

Not applicable.

Author Contribution

WJL contributed to the conception, design, analysis, and interpretation of the data, obtaining funding, and administrative, technical, or logistical support. SVS contributed to the drafting of the article, critical revision of the article for important intellectual content, final approval of the article, provision of study materials or patients, statistical expertise, and obtaining funding. JPS contributed to obtaining funding, administrative, technical, or logistical support, and provision of study materials or patients.

REFERENCES

Afandi, D., Aufi, F. R., Widyaningsih, C., et al. 2021. Correlation of head circumference with height based on sex in mongoloid race at 18-45 years old in Riau Province. Al-Iqra Medical Journal (Jurnal Berkala Ilmiah Kedokteran), 4(2): 64–70. doi: 10.26618/aimj.v4i2.6471.

Bharti, D., Gajrani, A., Singh, A. P. 2019. Relationship between height and head circumference in Haryanvi population in Panchkula region. Journal of Emerging Technologies and Innovative Research (JETIR), 6(5): 233–237. Available at: https://www.jetir.org/view?paper=JETIR1905I36.

Dahlan, M. S. 2021. Statistics for medicine and health 6th edition. Jakarta: Epidemiologi Indonesia. Available at: https://perpustakaan.umegabuana.ac.id/index.php?p=show_detail&id=2157&keywords=.

Eboh, D. E. O., Ohaju-Obodo, J. O. 2019. Height estimation from head dimensions in South-South Nigeria. Egyptian Journal of Forensic Sciences, 9(1): 62. doi: 10.1186/s41935-019-0159-x.

Elpeze, G., Usgu, G., Yiğit, S. 2023. Reliability of the smartphone application inclinometer and flexicurve in measuring thoracic kyphosis. Cureus, 15: 3. doi: 10.7759/cureus.35886.

Febriawan, R. 2015. Effect of head circumference on body height. Universitas Sebelas Maret. Available at: https://digilib.uns.ac.id/dokumen/detail/50205/Pengaruh-Lingkar-Kepala-terhadap-Tinggi-Badan.

Graha, G. K. 2023. Indonesian cultural diversity in the spirit of nationalism, Garda Timur Indonesia.

Available at: https://gti.or.id/2023/04/30/keberagaman-budaya-indonesia-dalam-semangat-nasionalisme/
IBM Corp. 2017. IBM SPSS statistics for Windows, version 25.0. Armonk, NY: IBM Corp. Available at: https://www.ibm.com/support/pages/downloading-ibm-spss-statistics-25.
Illahi, A. W. 2021. Sasi tradition: Marine ethnoconservation in the Cenderawasih Bay National Park Area, Forestation FKT UGM. Available at: https://forestation.fkt.ugm.ac.id/2021/05/30/tradisi-sasi-etnokonservasi-laut-di-kawasan-taman-nasional-teluk-cenderawasih/.
Imran, M. F. 2015. Mutilation in Indonesia. Jakarta: Yayasan Pustaka Obor Indonesia. Available at: http://obor.or.id/mutilasi-di-indonesia-modus-tempus-locus-actus.
Jayani, D. H. 2021. As many as 44.75% of the population of North Sumatra are Batak, databoks. Available at: https://databoks.katadata.co.id/datapublish/2021/10/07/sebanyak-4475-penduduk-sumatera-utara-bersuku-batak#:~:text=Silakan login untuk mengakses informasi ini.&text=Daftar sekarang%2C GRATIS!&text=Badan Pusat Statistik (BPS) melaporkan,dan 0%2C73%25 Pakpak.
Krishan, K., Kanchan, T., DiMaggio, J. A. 2010. A study of limb asymmetry and its effect on estimation of stature in forensic casework. Forensic Science International, 200(1–3): 181.e1-181.e5. doi: 10.1016/j.forsciint.2010.04.015.
Larasati, A. H., Koesbardiati, T., Yudianto, A. 2018. Estimation of body height based on head size in the mongoloid race in Pandean, Surabaya. Jurnal Biosains Pascasarjana, 20(2): 107. doi: 10.20473/jbp.v20i2.2018.107-119.
Mansur, D. I., Haque, M. K., Sharma, K., et al. 2014. Use of head circumference as a predictor of height of individual. Kathmandu University Medical Journal, 12(2): 89-92. doi: 10.3126/kumj.v12i2.13651.
Microsoft Corporation. 2016. Microsoft Excel. Available at: https://office.microsoft.com/excel.
Nabila, E. 2020. The effectiveness of a scoliometer as an early detection tool for scoliosis. Health & Medical Journal, 2(1): 58–61. doi: 10.33854/heme.v2i1.297.
National Disaster Management Authority. 2022. BNPB verifies 5,402 disaster events throughout 2021 National Disaster Management Authority (NDMA). Available at: https://bnpb.go.id/berita/bnpb-verifikasi-5-402-kejadian-bencana-sepanjang-tahun-2021.
National Disaster Management Authority. 2023. Tracing tsunami Aceh on 2004, National Disaster Management Authority (NDMA). Available at: https://sejarah.dibi.bnpb.go.id/story-map/Menelusuri-Tsunami-Aceh-2004/47.
Poluan, B., Tomuka, D., Kristanto, E. G. 2016. Relationship between head height and body height for forensic identification. Journal e-Clinic (eCl), 4(1): 1–3. Available at: https://ejournal.unsrat.ac.id/v3/index.php/eclinic/article/view/12110/11691.
Prenetha, R., Babu, K. 2022. Stature estimation using head circumference. Journal of Advanced Pharmaceutical Technology & Research, 13(5): 140. doi: 10.4103/japtr.japtr_217_22.
Putri, D. L., Kurniawan, R. F. 2022. Today in history: Remembering 16 years of the Yogyakarta earthquake 27 May 2006, Kompas.com. Available at: https://www.kompas.com/tren/read/2022/05/27/073358965/hari-ini-dalam-sejarah-mengenang-16-tahun-gempa-yogyakarta-27-mei-2006?page=all.
Reddy, M., Reddy, V., Wadhwan, V., et al. 2018. Correlation and estimation of stature from cephalofacial measurements: A study on Western Uttar Pradesh population. Journal of Forensic Dental Sciences, 10(2): 101. doi: 10.4103/jfo.jfds_30_16.
Romdhon, A. 2015. Reconstructive forensic identification using cephalometry index. Majority, 4(8): 23–28. Available at: https://id.scribd.com/document/341042817/23-28-ABDUL-RR-1.
Safitri, R. N., Rahmanto, S., Rahim, A. F. 2020. The relationship between a bent working position and the incidence of kyphosis in rice farmers in Grobogan Village. Universitas Muhammadiyah Malang. Available at: https://id.scribd.com/document/482181322/NASPUB-RISMA-pdf.
Sanders, M. S., McCormick, E. J. 1976. Human factors in engineering and design. McGraw-Hili. Available at: https://eprint.ulbi.ac.id/1712/1/Human Factor In Engineering And Design.pdf.
Saputri, A. H. N. S., Junitha, K. I., Suaskara, I. B. M. 2023. Forensic identification based on primary and secondary examinations as a determinant of victim identity. Simbiosis, 11(1): 1–14. doi: /10.24843/JSIMBIOSIS.2023.v11.i01.p01.
Shah, R. K., Kanani, S. K. D., Patel, J. P., et al. 2017. Estimation of stature from head circumference in Gujarati adolescent population. National Journal of Clinical Anatomy, 6(2): 146–151. doi: 10.1055/s-0039-1700739.

Shaw, M., Adam, C. J., Izatt, M. T., et al. 2012. Use of the iPhone for Cobb angle measurement in scoliosis. European Spine Journal, 21(6): 1062–1068. doi: 10.1007/s00586-011-2059-0.
Tur, J. A., Bibiloni, M. del M. 2019. Anthropometry, body composition and resting energy expenditure in human. Nutrients, 11(8): 1891. doi: 10.3390/nu11081891.

ORIGINAL RESEARCH REPORT

Effect of Virgin Coconut Oil Supplementation on AFB Sputum Conversion Rate, SOD, and BMI Levels in Pulmonary Tuberculosis Patients

Muhammad Ajib Nuzula*, Mohamad Isa, Juhairina, Haryati, Isa Ansori, Erna Kusumawardhani

Department of Pulmonology and Respiratory Medicine, Faculty of Medicine Lambung Mangkurat University, Ulin General Hospital Banjarmasin, Indonesia.

Article Info

Article history:
Received 16-03-2024
Revised 11-06-2024
Accepted 19-06-2024
Published 01-07-2024

Keywords:
Lung TB
VCO
AFB sputum
SOD
BMI
Malnutrition

***Corresponding author**:
Muhammad Ajib Nuzula
ajib.keren.2011@gmail.com

ABSTRACT

Background: Pulmonary tuberculosis has the potential to raise illness and death rates on a global scale significantly. A low sputum conversion rate for acid-fast bacilli (AFB) and malnutrition are indicators of failure in pulmonary TB treatment. Virgin coconut oil (VCO) contains lauric acid and has antibacterial, antioxidant, and anti-inflammatory effects. **Objective:** This study aimed to examine the effect of VCO supplementation on sputum AFB conversion rates, superoxide dismutase (SOD) levels, and body mass index (BMI) status of pulmonary TB patients. **Material and Methods:** This study was an analytic experimental study measuring BMI levels and SOD levels at the Pekauman Community Health Center, Sultan Suriansyah Hospital, and Ulin Hospital, Banjarmasin, Indonesia, between July and September 2023. This study, 40 samples were separated into two groups: the treatment group, which received first-line antithyroid drugs (ATD) with VCO, and the control group, which received first-line ATD and a placebo. **Results:** Based on the proportion, there was an improvement in the acceleration of BTA sputum conversion, and from the mean BMI and the median value of both groups, there was a decrease in SOD levels after VCO administration. No statistically significant association was found between VCO administration and accelerating the AFB sputum conversion and BMI at the end of the fourth and eighth weeks ($p>0.05$). Additionally, there was no relationship considered between VCO administration and reducing SOD levels before and after treatment ($p>0.05$). **Conclusion:** VCO administration had no relationship with the level of AFB sputum conversion, reduction in SOD levels, and BMI status in pulmonary TB patients.

How to cite:
Nuzula, M. A., Isa, M., Juhairina., et al. 2024. Effect of Virgin Coconut Oil Supplementation on AFB Sputum Conversion Rate, SOD and BMI Levels in Pulmonary Tuberculosis Patients. Majalah Biomorfologi-Biomorphology Journal, 34(2): 102-113.

Majalah Biomorfologi (Biomorphology Journal) p.ISSN:0215-8833, e.ISSN: 2716-0920
doi: 10.20473/mbiom.v34i2.2024.102-113

Highlights

1. VCO can accelerate AFB sputum conversion in tuberculosis infection.
2. VCO can decrease SOD levels as a marker of inflammation.

BACKGROUND

Tuberculosis (TB) has existed for thousands of years but remains a significant health issue in most countries with low incomes. The World Health Organization (WHO) reported 10.6 million new cases of tuberculosis in 2021, an increase of 600,000 cases from 2020, which was anticipated to be 10 million cases, with a mortality rate of more than 1.6 million (World Health Organization, 2022). Indonesia has the highest prevalence of TB cases among all countries. The country ranks second globally with a total of 969,000 cases and an incidence rate of 354 cases per 100,000 population (Firdaus, et al., 2023). In 2022, the South Kalimantan Provincial Health Service recorded a total of 7556 cases, out of which 3861 (51%) were confirmed to be positive for acid-fast bacilli (AFB) in sputum samples. Among these cases, Banjarmasin City accounted for 1768 cases, with 819 (46%) being positive for AFB sputum (Sukarli & Mahdani, 2023).

The high mortality rate resulting from failure to treat pulmonary TB is a concern. An important thing in evaluating treatment is assessing the conversion of AFB sputum. Sputum conversion in pulmonary TB cases occurs at the end of the first month (60-80%) and at the second month (95%). However, around 9% of cases do not experience sputum conversion (Aliyah, et al., 2016). AFB sputum conversion is a guideline for determining treatment success with a minimum rate of 80% (Mahendrani, et al., 2020).

Virgin coconut oil (VCO) has gained significant popularity and is extensively advocated and utilized for its health benefits. Coconut oil can also be referred to as tropical oil or lauric oil. VCO is natural oil due to its production procedure, which does not include heating. VCO possesses antibacterial, antioxidant, and anti-inflammatory properties (Bhatt, et al., 2021; Mela & Bintang, 2021). Nutritional status is also a determining factor in the success of pulmonary tuberculosis treatment based on measuring body mass index (BMI). The sputum conversion rate in patients with a BMI <18.5 kg/m^2 was lower than in patients with a BMI >18.5 kg/m^2 (Sari, et al., 2019).

The efficacy of therapy is also impacted by internal factors, one of which is oxidative stress (SO). SO occurs due to an imbalance between free reactive oxygen species (ROS) and antioxidant mechanisms, which play a crucial role in the inflammatory process in TB patients. Pulmonary tuberculosis carries a significantly higher chance of developing significant organ damage compared to other organs (Shastri, et al., 2018). Antioxidants in VCO, such as polyphenols, have been studied to prevent and treat diseases caused by oxidative damage. This polyphenol content can increase endogenous antioxidants, the SOD (Ighodaro & Akinloye, 2018).

OBJECTIVE

The main objective of this research was to determine the effects of VCO supplementation on sputum AFB conversion, SOD levels, and BMI in patients with pulmonary tuberculosis. The primary objective of this study was to examine the differences in AFB seroconversion, SOD level, and BMI following the administration of VCO supplementation at the end of the 4^{th} and 8^{th} week of the intervention.

MATERIAL AND METHOD

This study was analytic experimental research conducted on TB patients who were confirmed bacteriologically by examination of sputum AFB and TCM, SOD, and BMI levels at the Pekauman Community Health Center, Sultan Suriansyah Regional Hospital, and Ulin Regional Hospital, Banjarmasin, Indonesia. The research samples were all pulmonary TB patients diagnosed bacteriologically with positive AFB sputum and TCM MTb detected sensitive rifampicin who were examined and treated at the Pekauman Community Health Center, Sultan Suriansyah Regional Hospital and Ulin General Hospital Banjarmasin from July to September 2023, based on specific inclusion and exclusion criteria.

Inclusion criteria for this study included patients who were over 18 years of age and had signed informed consent, patients who were diagnosed with pulmonary TB based on positive AFB sputum results and sensitive rifampicin-detected TCM MTb who had not undergone first-line ATD treatment, TB patients were domiciled in the city of Banjarmasin, TB patients body weight and body height.

Exclusion criteria in this study were domiciled outside Banjarmasin, the patients who had undergone TB treatment for less than one month, patients who did not follow the research protocol in an orderly way, and patients who experienced severe side effects.

Sampling technique

The research sample was selected using simple random sampling in compliance with the inclusion and exclusion criteria. The study's sample was divided into two groups: patients receiving first-line ATD as a placebo control and those receiving first-line ATD with a dose of 1x30 ml VCO as the treatment group. The research was comparative analytic research using an unpaired numerical measurement scale from Dahlan, (2011):

$$S^n = \frac{S_1^2(n_1 - 1) + S_2^2(n_2 - 1)}{(n_1 + n_2 - 2)}$$

Legend:

N_1 = first group based on literature.
N_2 = second group based on literature.
S_1 = Group one standard deviation based on literature.
S_2 = Group two standard deviation based on literature

$$S^2 = \frac{S_1^2(n_1-1)+ S_2^2(n_2-1)}{n_1 + n_2 - 2}$$
$$S^2 = \frac{4^2(20-1)+6^2(20-1)}{20 + 20 - 2}$$
$$S^2 = \frac{16(19)+36(19)}{38}$$
$$S^2 = \frac{304+684}{38}$$
$$S^2 = \frac{988}{38}$$
$$S^2 = 26$$
$$S = \sqrt{26}$$
$$S = 5{,}09$$

So, the sample size is calculated using the formula above, using a hypothetical 5% alpha.

The research instruments were informed consent, a blood chemistry tube, a 3-cc injection syringe (One Med), a cooling box, a fridge or freezer, an Eppendorf 5702 brand centrifuge, sputum pot, glass objects, Ziehl Nielsen staining (Bio Analitika-3729298000-AL2-119592672), a spiritus lamp, a light microscope (Mikroskop Cahaya-3899601999-PP2-194217438), scales and meters (GEA), and liquid VCO (VCO TJ PT. Byhaf).

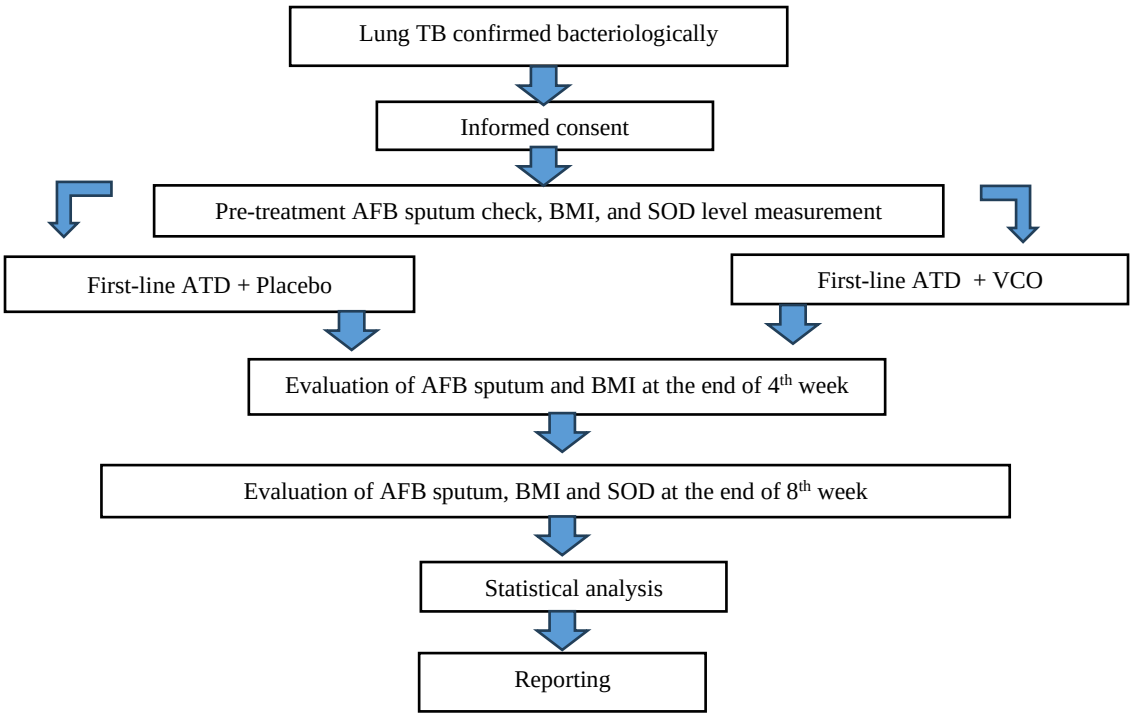

Figure 1. Flow chart of the research.

Patients who were suspected of having pulmonary tuberculosis were evaluated for AFB and TCM. If TCM MTb is detected and AFB is positive, it was included in the examined sample group. Afterward, informed consent was provided. The patients provided blood samples of 3 cc each, which were then subjected to centrifugation to separate the serum. The obtained serum was after that sent to the Banjarbaru biochemistry laboratory to measure the SOD levels. Then, the patients had a weight and height measurement to asses their Body Mass Index (BMI). During therapy for pulmonary tuberculosis, the patients were administered 30 cc of VCO daily for two months. The BW and height examination was conducted at the end of the first month of TB treatment and additional VCO supplementation. At the end of the second month, the patients were examined for BTA sputum, blood BW, and height. A 3cc blood sample was taken from the fold of the arm, and the serum was taken to check the SOD levels at the Banjarbaru biochemistry laboratory. Then, the data were subjected to statistical analysis, and a report was made.

Data analysis

The data were analyzed using the IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, N.Y., USA). The data had previously been tested for homogeneity and normality. The initial data were analyzed using univariate analysis, which used descriptive statistics to show normally distributed values as mean and standard deviation and non-normally distributed values as median and range. Bivariate analysis was conducted utilizing parametric tests and the independent t-test to quantify SOD and BMI levels. The Mann-Whitney test was employed when the data distribution was not normal ($p< 0,05$). The Chi-Square test was also used to analyze sputum conversion ($p> 0,05$). Fischer exact test was employed if the expected count value was <5 and >20 ($p> 0,05$).

Ethical approval

Ethics permission was obtained with the approval and consideration of the Ethics Commission of the Faculty of Medicine, Lambung Mangkurat University (No. 314/KEPK-FK ULM/EC/X/2023) on 02-10-2023, and the Ethics Commission of the Research and Development Agency of ULIN Hospital Banjarmasin (No. 162/VII-Reg Riset/RSUDU/23) on 25-07-2023.

RESULT

Subject characteristic

Table 1 describes data on age, gender, smoking, type of cigarette, Brinkman index, length of time to stop smoking, comorbidities, occupation, body weight, nutritional status, and side effects after two months of treatment.

Table 1. Sample characteristics in treatment and control groups.

Sample characteristics		Data	
		DOTS+VCO (n=20)	DOTS+ Placebo (n=20)
Gender	Male (%)	14(70)	12(60)
	Female (%)	6(30)	8(40)
Age	18-24 (yo)	3(15)	4(20)
	25-34 (yo)	2(10)	3(15)
	35-44 (yo)	3(15)	6(30)
	45-54 (yo)	6(30)	1(5)
	>55 (yo)	6(30)	6(30)
Smoking	Smoker (%)	14(70)	12(60)
	Not smoker (%)	6(30)	8 (40)
Brinkman index	Mild (%)	6(42,8)	3(25)
	Moderate (%)	7(50)	7(58,3)
	Severe (%)	1(7,1)	2(16,6)
Comorbidities	Hypertension (%)	3(15)	2(10)
	Diabetes (%)	4(20)	5(25)
	Hypertension and diabetes (%)	2(10)	0
	COPD (%)	0	1(5)
	HIV (%)	0	1(5)
	No comorbidity (%)	11(55)	11(55)
Weight	Before treatment (mean)	45.45	50.12
	End of 4th week (mean)	47.17	51.35
	End of 8th week (mean)	49.15	53.5
Good nutrition	Before treatment (mean)	8(40)	10(50)
	End of 4th week (mean)	10(50)	10(50)
	End of 8th week (mean)	14(70)	11(55%)
Malnutrition	Before treatment (mean)	12(60)	10(50)
	End of 4th week (mean)	10(50)	10(50)
	End of 8th week (mean)	6(30)	9(45)
Side effects	Nausea	7(35)	0
	Diarrhea	2(10)	0

Legend: SD: Standard deviation.

Table 1 shows that the most common gender in the treatment and control groups is male, with 14 individuals (70%) in the treatment group and 12 individuals (70%) in the control group. Meanwhile, for age characteristics in the control group, it was found that the highest age range was 35-44 years (30%) and age >55 years (30%), while in the treatment group, the age range of 45-54 years and >55 years were dominated by 30% each.

According to smoking data history, 12 (60%) samples of the control group smoked, and 8 (40%) samples of non-smokers. Meanwhile, in the treatment group, data were obtained that 14 (70%) people smoked and 6 (30%) people did not smoke. The Brinkman index found that most of the control group were moderate smokers (58.3%), followed by light smokers (25%) and heavy smokers (16.6%). In the treatment group, it was also found that the majority were moderate smokers (50%), followed by light smokers (42.8%) and finally heavy smokers (7.1%).

In the treatment group, the most prevalent comorbidity among study subjects was diabetes mellitus (DM), with a rate of 4 people (20%). In the control group, DM occurred in 5 people (25%). Hypertension ranked second in both groups, with three people (15%) in the therapy group and two (10%) in the control group. The combined number of individuals with comorbidities in both the treatment and control groups was nine people each, accounting for 45% of the total.

Table 1 shows that the control group (first-line ATD + placebo) and the treatment group (first-line ATD + VCO) had weight gain. However, it was seen that the placebo group had a higher mean BW compared to the treatment group. In Table 1, the nutritional status of the treatment group in the good category experienced a significant increase from 8 people (40%) to 10 people (50%). At the end of the 8th week, it increased to 14 people (70%). The malnutrition category decreased significantly from 12 people (60%) to 10 people (50%) at the end of the 4th week, and also at the end of the 8th week it decreased to 6 people (30%).

The control group did not experience any adverse effects due to the placebo administration. In the meantime, the treatment group experienced two adverse effects: diarrhea and nausea. Nausea was the most prevalent adverse effect in 35% of the treatment group samples, while diarrhea was observed in 10% of the treatment group.

Table 2. BMI normality tests.

Normality test	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
IMT 0	.131	40	.082	.960	40	.171*
IMT 1	.106	40	.200*	.977	40	.582*
IMT 2	.148	40	.028	.950	40	.078*
SOD 1	.210	40	<.001	.909	40	.004
SOD 2	.084	40	.200*	.970	40	.361*

Table 2 shows that the Shapiro-Wilk test is carried out for BMI status in the treatment and control groups because the sample size is <100 patients. The data before the intervention, in the first and fourth week, showed p>0.05, so the sample distribution was normal and met the requirements for the unpaired t-test to be carried out further.

The research subject data were tested for normality on SOD levels. We carried out the Shapiro-Wilk test because the sample size was <100 patients to assess the normality of data distribution. The data would be normally distributed if the significance value is (p>0.005). Based on the data from the normality test, results on SOD levels before treatment revealed p=0.004 (p<0.05), so the SOD data before treatment were not normally distributed. Whereas SOD levels after the eighth week of treatment showed p=0.361 (p>0.05), indicating normally distributed data.

Variable characteristics

The variable characteristics of this study were the AFB sputum conversion rate and changes in BMI status at the end of the 4th and 8th weeks as well as changes in SOD levels before and after eight weeks in the treatment and control groups.

Table 3 shows that ten patients (50%) in the control group who were given a placebo converted to a negative AFB sputum at the end of the 4th week, and the remaining ten patients (50%) had not experienced conversion. Meanwhile, at the end of the 8th week, 17 patients experienced negative conversions (85%), and three failed to convert (15%). In the treatment group receiving VCO, there was a conversion of AFB sputum to negative at the end of 4th week. As many as 16 patients (80%) and the remaining four (20%) had not experienced conversion. Meanwhile, at the end of the 8th week, 20 patients (100%) experienced negative conversions. Chi-square analysis of conversion status after four weeks of ATD treatment in the control group and treatment group receiving VCO supplementation (Table 2) reveals no significant difference in conversion status with p=0.110. From the results of the tabulation of the chi-square test on AFB sputum conversion at the end of the eighth week, it was found that two cells had an expected count of < 5, so a Fisher exact test was carried out, and the results were p=0.231 (p>0.05).

Table 3. Variable characteristics in treatment and control groups.

Variable characteristics		Data		p
		ATD+ VCO (n=20)	ATD+Plasebo (n=20)	
AFB sputum conversion	End of 4th week (%)	16(80)	10(50)	0.110
	End of 8th week (%)	20(100)	17(85)	0.231
BMI	Before treatment (mean±SD)	17.53±3.33	19.04±3.05	0.614
	End of 4th week (mean±SD)	18.48±3.56	19.29±3.33	0.872
	End of 8th week (mean±SD)	19.32±3.62	20.12±4.08	0.430
SOD level	Pre (median[min-max])	0.019 (0.001-0.033)	0.017 (0.003-0.03)	0.114
	Post (median[min-max])	0.013 (0.000-0.031)	0.0135 (0.000-0.025)	0.989

Legend: BMI: Body mass index; SOD: superoxide dismutase; AFB: Acid-fast bacilli; SD: Standard deviation.

SOD levels in the treatment group before treatment had a median of 0.019 (0.001-0.033) decreased to 0.013 (0.000-0.031) at the end of the 8th week. SOD levels in the control group before treatment had a median of 0.017 (0.003-0.03) decreased to 0.0135 (0.000-0.025) at the end of the 8th week. Based on the non-parametric test analysis with the Man Whitney test in Table 3, the results obtained before treatment have p=0.114, and after administration of VCO, the p-value is 0.989 (p>0 .05).

The mean BMI value in the control group increased from 19.04 ± 3.05 kg/m^2 before intervention to 19.29 ± 3.33 kg/m^2 at the end of the 4th week and again to 20.12 ± 4.08 kg/m^2 at the end of the 8thweek. The mean BMI value in the treatment group increased from 17.53 ± 3.33 kg/m^2 before the intervention to 18.48 ± 3.56 kg/m^2 at 4th week and again to 19.32 ± 3.62 kg/m^2 at the end of the 8th week. From the results of the unpaired t-test on BMI status before treatment, the p-value was 0.614. Then, at the end of the 4th week, the BMI status had p=0.878, and at the end of the 8th week, the BMI status had p=0.43 (Table 3).

DISCUSSION

Several studies showed that VCO was beneficial against TB. In pulmonary TB patients who are administered first-line ATD in conjunction with VCO tablets, the conversion of AFB sputum can be expedited. The content of a long chain of saturated fat known as glyceryl monolaurate, which functions as a potent bactericide for pulmonary tuberculosis, accelerates the conversion (Djannah, 2022).

Sample characteristic

In 2020, based on WHO data, it is estimated that 5.6 million men (56%) and 3.3 million women (33%) will be infected with MTb germs. Based on data from the Ministry of Health regarding the total number of tuberculosis cases, tuberculosis cases in men more than in women both in 2021 (men 57.7%; women 42.3%) and 2022 (men 57.8%; women 42.2%) (Sukarli, 2022). This aligns with this research where the majority were men in the placebo group, as many as 12 patients (60%), and 14 patients (70%) in the treatment group.

Age is a unit of time in a person's life from birth to the present. The age classified as unproductive is 58 years, while the productive is 15–58 years. The productive age refers to the period in a person's life when they are most likely to interact frequently with others in educational, professional, or other social settings (Novita, et al., 2022). TB sufferers in productive age reached 75%, while the rest occurred in non-productive age (Novita, et al., 2022). The data presented aligns with the findings of this study, indicating that the majority of individuals in the research sample were between the ages of 45 and 54 and >55 years old, accounting for 30% each in the treatment group. Similarly, in the control group, individuals aged 35-44 and >55 years old accounted for 30% each.

Cigarettes contain 4500 harmful substances that enter the body and damage some of the lung's defense mechanisms, which can interfere with mucosal cleansing and decrease the function of alveolar macrophages to phagocytose bacteria (Velayati & Farnia, 2016). The research revealed that 26 individuals (65%) from the 40 research samples smoked. The treatment group consisted of 14 patients, while the control group consisted of 12 patients. Therefore, it was hypothesized that the immune system declined since many of the research subjects smoked, resulting in the discussion of the healing process from pulmonary TB.

In this study, the majority (58.3%) had a Brinkman index of 200-599, indicating that there were moderate smokers, and there were 17.1% of heavy smokers with a Brinkman index of more than 600 in the control group. Meanwhile, in the treatment group, the Brinkman index was lower, with 50% moderate smokers and 7.1% heavy smokers. The toxins caused by smoking accumulate in the body over time, causing more dangerous consequences (Maqfirah, et al., 2020).

Comorbidities associated with the development of tuberculosis and poor outcomes are HIV infection, diabetes mellitus (DM), kidney disease, and smoking, as described in Figure 2. Diabetes, smoking, and HIV infection are known to influence the metabolic state of the host, macrophages, and systemic levels (Llibre, et al., 2021). In this study, there was one sample (2.5%) who had comorbid HIV in the control group, and nine samples had comorbid DM (22.5%). In TB patients, the prevalence of DM after screening ranges from 1.9% to 35%, with the highest rates in countries with high diabetes rates (Fadillah, et al., 2021).

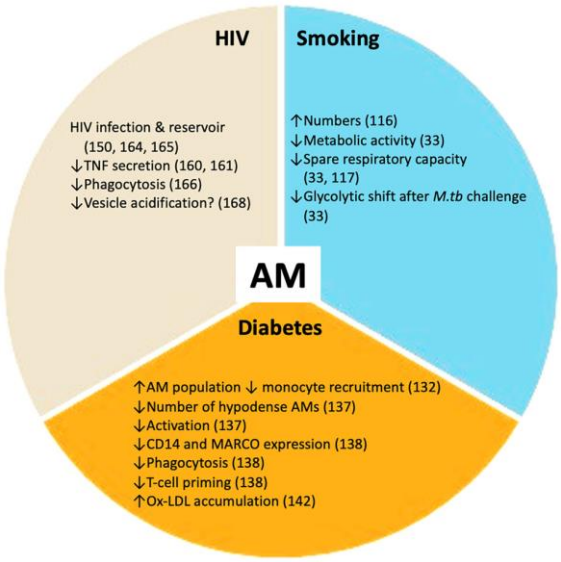

Figure 2. Comorbid in tuberculosis affect on alveolar macrofag. smoking, HIV infection, and diabetes disturbed alveolar macrophage phenotype (Bahtiar, 2021).

Relationship between VCO and AFB sputum conversion improvement

At the end of the 4th week, the Chi-Square test tabulation results indicated no relationship between VCO administration and AFB sputum conversion, as indicated by p=0.110 (p>0.05). Similarly, the Chi-square test results on AFB sputum conversion at the end of the 8th week revealed that two cells had an expected count of <5, necessitating a Fisher exact test. The results were p=0.231 (p>0.05), indicating no relationship between VCO administration and, at the end of the 8th week, an increase in the rate of AFB sputum conversion. There was no significant increase in AFB sputum conversion in the 4th and 8th weeks after VCO administration. This might have been because of factors that made the results unclear, such as smoking, the level of bacteria in AFB sputum, and having DM simultaneously. In numerous studies, smoking is an independent predictor of changes in sputum conversion. Smoking suppresses the lung's defense mechanisms against infection (Anandaraj, et al., 2017).

Administering VCO has a positive effect on increasing the conversion of AFB sputum. Sputum conversion is a strong predictor of the effectiveness of TB treatment. AFB sputum conversion was 60–80% in the first month and 95% in the second month (Dewi, 2019). In this study, the conversion results

in the first month for controls who received a placebo were 45% lower than in previous studies. Compared to previous studies (Djannah, 2022), those who received VCO converted 70% less AFB sputum. Meanwhile, in the second month of the study, the control conversion rate was 85%, which was lower than the previous study (Djannah, 2022), and those who received VCO resulted in 100%, which was higher than the earlier research findings.

At the end of the fourth week, 45% of the AFB sputum conversion failure rate was still positive for 1, and 5% was positive for 2 cases. Then, at the end of the eighth week, 15% of those who failed to convert were three patients. This indicated that these three individuals' bacterial count remains elevated, and this patient had comorbid DM. This was based on the hypothesis that the immune system's impairment in patients with TB and DM leads to a decrease in the activation of alveolar macrophages and the ability to produce interleukins, resulting in a delayed conversion of AFB sputum (Anandaraj, et al., 2017). In this study, the comorbid condition of DM was controlled with regular treatment.

The relationship between VCO and reducing SOD levels

Chattopadhyay's, (2019) study measured serum SOD activity in pulmonary TB patients treated with first-line ATD for 0–15 days and then one month after the next ATD. Pulmonary and extrapulmonary TB patients had significantly increased SOD levels before treatment, which can be used as an early marker of MTb infection. Serum SOD was reduced by 31% in pulmonary TB patients and by 29% in extrapulmonary TB patients after one month of first-line ATD. In some patients, SOD levels may remain elevated during the first month of treatment due to drug resistance. Consequently, some patients must respond to first-line ATD (Chattopadhyay, 2019).

The results of the Man-Whitney test in this study were $p>0.05$, indicating that there was no statistically significant difference in the administration of VCO to reduce SOD levels in pulmonary TB patients. The absence of statistical significance may be attributed to the presence of factors that affect oxidative stress in pulmonary TB patients, such as hypertension, diabetes mellitus, and smoking behaviors (Cardoso, et al., 2015).

At the end of the eighth week, the treatment group experienced a proportional decrease in SOD levels compared to before the VCO treatment. This was due to a reduction in the oxidative stress process, considered a marker of recovery for pulmonary TB patients, and a decrease in the virulence of MTb germs. The pulmonary tuberculosis infection process resulted in elevated intracellular oxidative stress conditions, leading to higher SOD levels before treatment than after treatment.

The effect of VCO on nutritional status

The unpaired T-test analysis found $p>0.05$, so there was no relationship between VCO administration and increasing BMI levels in the control and treatment groups before the intervention at the end of the 4th and 8th weeks. This was due to the non-homogenized nutritional status in the study, indicating that not only the micronutrient content of VCO but also multifactorial factors such as nutritional intake could improve nutritional status (Cendhikalistya & Makiyah, 2009; Iswati & Nuraini, 2023).

In this study, there was a proportional increase in nutritional status in the treatment group, from poor dietary status to good nutrition after VCO administration. Poor nutritional status raises the risk of developing pulmonary TB. After the intervention in the 4th week, the situation remained unchanged, with 50% of the sample experiencing malnutrition, which improved to only 45%. Meanwhile, 60% of the sample in the treatment group was malnourished. After the fourth week, it rose to 50% of those underweight. In the 8th week, it was found that 30% were still malnourished. As a result, the effect of VCO on nutritional status tends to increase compared to controls.

Strength and limitations

The study's strength lies in the proportional increase in AFB sputum conversion speed between the treatment and control groups at the end of the 4th and 8th weeks. In proportion, the treatment group's mean BMI increased from malnutrition to adequate nutrition at the end of the 4th and final week of the 8th, compared to the control group. At the end of week 8, there was a more significant reduction in SOD levels in the treatment group than in the control group, according to the median value.

The study's limitation was that statistical tests were not performed for confounding variables. So, it was not straightforward to see whether confounding variables in this study could have influenced the results. In this study, the antioxidant level was only SOD. In contrast, other endogenous antioxidants,

such as glutathione peroxidase and catalase, were not used, but they also have a role in fighting oxidative stress. Apart from that, this study did not use radiological images related to bacteriology. The lack of homogenization for comorbid conditions could be a weakness. Homogenization of nutritional status classification is still needed.

CONCLUSION

At the end of the 4th and 8th weeks, the treatment group increased the speed of AFB sputum conversion compared to the control group. There was also a shift in the mean BMI from malnutrition to good nutrition in the treatment group compared to the control group at the end of the 4th and final weeks. In the final week, there was a more significant reduction in SOD levels in the treatment group than in the control group, according to the median value.

When compared to the control group, there was no difference in the rate of AFB sputum conversion, SOD levels, or BMI values in pulmonary TB patients who were given virgin coconut oil.

Further research is needed to examine the influence of nutritional intake, smoking habits, gender, and comorbidities on AFB sputum conversion after VCO administration. It is also necessary to assess the severity of lung parenchymal damage, which can affect AFB sputum conversion. A more extensive research population is required for future research. It is best to homogenize comorbid conditions and nutritional status for future research.

Acknowledgment
We want to thank Pekauman Public Health Service, Sultan Suriansyah Hospital, and Ulin Hospital, Indonesia, for their help in this research.

Conflict of Interest
The authors declared there is no conflict of interest.

Ethic Consideration
Ethics permission was obtained with the approval and consideration of the Ethics Commission of the Faculty of Medicine, Lambung Mangkurat University (No. 314/KEPK-FK ULM/EC/X/2023) on 02-10-2023, and the Ethics Commission of the Research and Development Agency of ULIN Hospital Banjarmasin (No. 162/VII-Reg Riset/RSUDU/23) on 25-07-2023.

Funding Disclosure
None

Author Contribution
MAN contributes to the conception and design, analysis and interpretation of the data, drafting of the article, and critical revision of the article for important intellectual content and final approval of the article. MI contributes to the conception and design, collection and assembly of the data, drafting of the article and final approval of the article. J contributes to the analysis and interpretation of the data, drafting of the article, and final approval of the article. H contributes to the analysis and interpretation of the data and final approval of the article. IA contributes to the analysis and interpretation of the data and final approval of the article. EK contributes to the critical revision of the article for important intellectual content and final approval of the article.

REFERENCES

Aliyah, N., Pranggono, E. H., Andriyoko, B. 2016. Description of sputum conversion of Acid-Fast Bacteria (BTA) and Vitamin D in new cases of pulmonary tuberculosis patients. Indonesian Journal Chest & Critical Emergency Medicine, 3(1): 1–4. Available at: https://www.indonesiajournalchest.com/index.php/IJC/issue/view/68.

Anandaraj, R., Anurupa, M. S., Kavithai, P., et al. 2017. Factors influencing delay in sputum smear conversion among new smear-positive pulmonary tuberculosis patients of davangere tuberculosis unit. International Journal of Medical Science and Public Health, 6(11): 1565–1571. doi:

10.5455/ijmsph.2017.0824913092017.
Bakhtiar, M. I., Wiedyaningsih, C., Yasin, N. M., et al. 2021. Relationship between characteristics, compliance and clinical outcomes of pulmonary tuberculosis patients at the Bantul District Health Center. 2021. Majalah Farmaseutik, 17(2). doi: 10.22146/farmaseutik.v17i2.60681.
Bhatt, K., Pulickal, A. V., Agolli, A., et al. 2021. Antioxidant, antibacterial, antiviral and antifungal properties of virgin coconut oil (VCO). Journal of Nutritional Biology, 7(1). doi: 10.18314/jnb.v7i1.2090.
Cardoso, D. A., Moreira, A. S. B., de Oliveira, G. M. M., et al. 2015. A coconut extra virgin oil-rich diet increases HDL cholesterol and decreases waist circumference and body mass in coronary artery disease patients. Nutricion Hospitalaria, 32(5),: 2144–2152. doi: 10.3305/nh.2015.32.5.9642.
Cendhikalistya, G. O., Makiyah, S. N. N. 2009. The influence of VCO (virgin coconut oil) to body weight in rat (*Rattus norvegicus*). Mutiara Medika: Jurnal Kedokteran dan Kesehatan, 9(1): 33–41. doi: 10.18196/mmjkk.v9i1.1589.
Chattopadhyay, D. K. 2019. Superoxide dismutase: A biomarker for early diagnosis of tuberculosis. Journal of Clinical and Diagnostic Research, 13(7). doi: 10.7860/JCDR/2019/35298.12968.
Dahlan, M. S. 2011. Statistics for medicine and health. Jakarta: Salemba Medika. Available at: https://books.google.co.id/books?hl=id&lr=&id=Abh5OaO3qlMC&oi=fnd&pg=PA1&ots=SXRFIe22BW&sig=--AwuSGRVQ2T1a8D8JaGTlgmAkY&redir_esc=y#v=onepage&q&f=false.
Dewi, B. D. N. 2019. Diabetes mellitus & tuberculosis infection: Diagnosis and therapeutic approaches. Yogyakarta: ANDI. Available at: http://repository.wima.ac.id/id/eprint/27079/1/1-br-Diabetes_melitus_.pdf.
Djannah, F., Massi, M. N., Hatta, M., et al. 2022. Virgin coconut oil and tuberculosis: A mini-review. Pharmacognosy Journal, 14(2): 464-469. doi: 10.5530/pj.2022.14.59.
Fadillah, M. I., Nurrasyidah, I., Fajari, N. M. 2021. Literatur review: The influence of diabetes mellitus with AFB sputum conversion after intensive phase in lung tuberculosis patient. Journal Of Homeostasis, 4(1): 81-92. doi: 10.20527/ht.v4i1.3349
Firdaus, A., Ruhman, F., Nasution, R. 2023. TB case detection in 2022 highest on record: Ministry, Antara: Indonesian News Agency. Available at: https://en.antaranews.com/news/277353/tb-case-detection-in-2022-highest-on-record-ministry.
IBM Corp. 2019. IBM SPSS statistics for Windows, version 26.0. Armonk, NY: IBM Corp. Available at: https://www.ibm.com/support/pages/downloading-ibm-spss-statistics-26.
Ighodaro, O. M., Akinloye, O. A. 2018. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria Journal of Medicine, 54(4): 287–293. doi: 10.1016/j.ajme.2017.09.001.
Iswati, R. S., Nuraini, I. 2023. Effectiveness of virgin coconut oil (VCO) on changes in weight and height among under-five children with stunting. Embrio: Jurnal Kebidanan, 15(1): 112–118. doi: 10.36456/embrio.v15i1.7003.
Lawn, S. D., Zumla, A. I. 2011. Tuberculosis. The Lancet, 378(9785): 57–72. doi: 10.1016/S0140-6736(10)62173-3.
Llibre, A., Dedicoat, M., Burel, J. G., et al. 2021. Host immune-metabolic adaptations upon mycobacterial infections and associated co-morbidities. Frontiers in Immunology, 12. doi: 10.3389/fimmu.2021.747387.
Mahendrani, C. R. M., Subkhan, M., Nurida, A., et al. 2020. Analysis of factors that influence the conversion of sputum to acid-fast bacilli in tuberculosis sufferers. Al-Iqra Medical Journal : Jurnal Berkala Ilmiah Kedokteran, 1(2): 1–9. doi: 10.26618/aimj.v3i1.4037.
Maqfirah, M., Dangnga, M. S., Hengky, H. K. 2020. The effect of smoking on the incidence of sputum conversion in pulmonary tuberculosis patients in Parepare City. Jurnal Ilmiah Manusia dan Kesehatan, 3(2): 206–217. doi: 10.31850/makes.v3i2.301.
Mela, E., Bintang, D. S. 2021. Virgin coconut oil (VCO): Production, advantages, and potential utilization in various food products. Jurnal Penelitian dan Pengembangan Pertanian, 40(2):. 103–110. doi: 10.21082/jp3.v40n2.2021.p103-110.
Novita, E., Suryani, L., Siringoringo, H. E. 2023. Analysis od the incidence of pulmonary tuberculosis. Jurnal Kesehatan Saelmakers Perdana, 6(1):1-17. doi: 10.32524/jksp.v6i1.827
Sari, D. K., Mega, J. Y., Harahap, J. 2019. Nutrition status related to clinical improvement in AFB-positive pulmonary tuberculosis patients in Primary Health Centres in Medan, Indonesia. Open Access

Macedonian Journal of Medical Sciences, 7(10): 1621–1627. doi: 10.3889/oamjms.2019.338.

Shastri, M. D., Shukla, S. D., Chong, W. C., et al. 2018. Role of oxidative stress in the pathology and management of human tuberculosis. Oxidative Medicine and Cellular Longevity, 2018: 1–10. doi: 10.1155/2018/7695364.

Sukarli., Mahdani. 2023. South Kalimantan records 7,556 TB cases, strives to reduce, Antara Kalsel. Available at: https://kalsel.antaranews.com/berita/367716/south-kalimantan-records-7556-tb-cases-strives-to-reduce.

Sulistyo, Utami ASF, Wicaksono AB, Salsabila A, Karlinda AY, Lestari AA, et al 2022. 2021 Tuberculosis Control Program Report. Jakarta: Kementerian Kesehatan RI, 2022. ISBN 978-623-301-375-8. Available at: https://tbindonesia.or.id/wp-content/uploads/2023/02/Laporan-Tahunan-Program-TBC-2021_Final-20230207.pdf.

Velayati, A. A., Farnia, P. 2016. Atlas of mycobacterium tuberculosis. Elsevier. Available at: https://shop.elsevier.com/books/atlas-of-mycobacterium-tuberculosis/velayati/978-0-12-803808-6.

World Health Organization. 2022. Global tuberculosis report 2022, WHO. Available at: https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2022/tb-disease-burden/2-1-tb-incidence#:~:text=The estimated 10.6 million people,2.1.

ORIGINAL RESEARCH REPORT

The Effect of Laser-Assisted Hatching on Pregnancy Outcomes of Vitrification Frozen Embryo Transfer

Vellyana Lie[1,5*], Andri Rezano[1,2], Tono Djuwantono[3], Maitra Djiang Wen[4,5]
[1]Andrology Study Program, Faculty of Medicine, Universitas Airlangga;
Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.
[2]Department of Biomedical Science, Faculty of Medicine, Universitas Padjadjaran, Sumedang, Indonesia.
[3]Department Obstetrics and Gynecology, Faculty of Medicine, Universitas Padjadjaran;
Hasan Sadikin General Hospital, Bandung, Indonesia.
[4]Bocah Indonesia Fertility Center, Tangerang, Indonesia.
[5]Wahidin Sudiro Husodo General Hospital,Mojokerto, Indonesia.

Article Info
Article history:
Received 24-11-2023
Revised 08-05-2024
Accepted 13-05-2024
Published 01-07-2024

Keywords:
Zona pellucida
Zona thickness
Cryopreserved
Good health and well-being
FET

***Corresponding author**:
Vellyana Lie
vellyana.lie-2022@fk.unair.ac.id

ABSTRACT

Background: Zona Pellucida (ZP) thickness of less than 16 mm is better for embryo implantation inside the endometrium. Laser Assisted Hatching (LAH) is commonly used, especially in non-contact mode, using a 1.48-um infrared diode laser beam because of its short exposure time, accurate positioning, simple operation, indirect contact, safety, and effectiveness. **Objective**: This paper describes the potential of laser-assisted hatching in biochemical pregnancy outcomes in Frozen Embryo Thawed transfers. **Material and Method**: The total number of patients enrolled in this study was 141. Patients were prospectively treated during embryo transfers at Pusat Fertilitas Bocah Indonesia, Primaya Hospital at Tangerang, Indonesia, from December 2020 until December 2021. **Result**: There were no significant differences between the LAH and no-LAH groups regarding average age, infertility duration, infertility type, and etiology of infertility ($p>0.05$). In the same line, the blastocyst (0.76 ± 0.87; 1.25 ± 1.08) compared with cleavage (0.72 ± 0.84; 0.67 ± 0.98) ($p<0.534$ and $p< .032$). There was no significant difference, even though clinically, the proportions of live births, multiple pregnancies, biochemical pregnancies, and ongoing pregnancies in the LAH group were not exceptions to the outcomes of failures and miscarriages. **Conclusion**: LAH did not appear to increase the pregnancy rate in this study. However, the methodology seems to reduce bias in this study by considering methodology for selecting FET embryos based on the cryopreserved condition, embryo quality, and precise LAH.

How to cite:
Lie, V., Rezano, A., Djuwanto T., et al. 2024. The Effect of Laser-Assisted Hatching on Pregnancy Outcomes of Vitrification Frozen Embryo Transfer. Majalah Biomorfologi-Biomorphology Journal, 34(2): 114-122.

Majalah Biomorfologi (Biomorphology Journal) p.ISSN:0215-8833, e.ISSN: 2716-0920
doi: 10.20473/mbiom.v34i2.2024.114-122

Highlights

1. LAH might effectively increase pregnancy outcomes for frozen-thawed embryo transfer.
2. Zona pellucida drilling might help increase pregnancy outcomes in FET.

BACKGROUND

Zona Pellucida (ZP) is an extracellular coat surrounding mammalian growing oocytes, ovulated oocytes, and early embryos. During the secondary follicle stage of oogenesis, the zona is secreted. The thickening of the ZP begins during the growth stage as the diameter of the oocytes increases. The zona thickness of less than 16 mm is better for embryo implantation inside the endometrium. It also protects the ovum during oviduct transit and the oocyte and embryo during the early stages of development from infection, as well as maintaining blastomere structure during preimplantation development (Le, et al., 2018; Nieschlag, et al., 2023). The structure of the ZP changes after fertilization, making it impossible for sperm to adhere to it. Before uterine implantation may occur, an enlarged blastocyst must hatch outside from the ZP, so if the thickness of the ZP is more than 16 mm, it is hard to implant. Proteolytic sublysis of the ZP by the embryo or the dam's endometrial lining and hydrostatic pressure created by fluid accumulation in the developing blastocoel cavity are two processes that allow ZP rupture and blastocyst escape (Jeong, et al., 2018).

In IVF-embryo transfer, abnormalities of the hatching process have been blamed for poor implantation. Assisted hatching (AH) was proposed to foster spontaneous hatching and improve embryo implantation (Hammadeh, et al., 2011).

Many studies have investigated the use of AH procedures to treat patients with poor-quality embryos and endometrium as a consequence of advanced maternal age (Le et al., 2018). Four methods used in the artificial rupture of ZP were mechanical partial ZP dissection, chemical ZP drilling using acid solution or enzymes, and laser micromanipulation (Gerri, et al., 2020) These methods aim to create holes in the ZP.

LAH is commonly used, especially in non-contact mode, using a 1.48-um infrared diode laser beam because of its short exposure time, accurate positioning, simple operation, indirect contact, safety, and effectiveness. Similar studies reported that efficacy of ZP dissection and implantation rate among embryos hatched by LAH, acid Tyrode's solution, and pronase enzymes (Wang, et al., 2022; Wei, et al., 2023). In other study Tannus, et al., (2019) showed no benefit of the ZP thinning in frozen-thawed embryo transfer.

OBJECTIVE

This article aimed to describe the potential of LAH in biochemical pregnancy outcomes in Frozen EmbryoThawed (FET) transfers.

MATERIAL AND METHOD

Patients' characteristics

The total number of patients enrolled in this study was 141. Patients were prospectively treated during embryo transfers at Pusat Fertilitas Bocah Indonesia, Primaya Hospital at Tangerang, Indonesia, from December 2020 until December 2021. This method was repeated every other day throughout this study.

All patients met the following inclusion criteria: ≥25 years of age with indications for IVF-embryo transfer: male factor, female factor, unexplained infertility, and mixed factor. Informed consent was obtained from all women, and this investigation received the approval of the Ethics Committee of Primaya Hospital in Tangerang City dated 26-03-2022.

Fertilization and embryo culture

All patients were treated antagonist short protocol for ovarian stimulation (Siristatidis, et al., 2022). Oocyte retrieval was performed by ultra-sound-guided follicle aspiration 36–38 hours after hCG administration. The oocytes underwent standard IVF and ICSI and were cultured in G-1 version 3.0 (Vitrolife, Kungsbacka, Sweden), supplemented with 10% recombinant human serum albumin (rHA, Vitrolife) for two days. On the day of embryo transfer (44–48 h after sperm insemination or injection), the embryos were scored according to the following Sydney and Gardner criteria for embryos (Blank, et al., 2020; Stigliani, et al., 2021). In some patients, when there were no more than two good-quality embryos, one or two extra poor-quality embryos were also selected for the transfer. However, the

patients with all poor-quality embryos had poor endometrial response or had a ZP thickness of >16 mm and were excluded from the study.

Embryos were randomly divided into two groups. In the control groups, the selected embryos were intact before the intrauterine transfer, whereas in the test groups, they were subjected to LAH. The embryo transfer was performed with a Kitazato catheter, whereas the physician was blinded to the control and test groups. The luteal phase was supported by intravaginal (Cyclogest 400, in two divided doses, for 800 mg/d; Actavis, Barnstaple, United Kingdom), starting the artificial cycle decided.

The patients were tested for serum ß-hCG 50mIU/ml assay 15 days after embryo transfer (Maheshwari, et al., 2019). If the biochemical pregnancy test was achieved, patients were followed with serial ultrasound to determine fetal viability. Clinical pregnancy was defined as the presence of a gestational sac on transvaginal ultrasound. When pregnancy occurred, luteal support was continued until 12 weeks gestation.

Freezing and thawing

The embryos were frozen using previously established protocols (ultra-rapid freezing). For thawing, the embryos were transferred from the culture media to the basal media (BM) containing HEPES (Quinn's SAGE, ART-1024) and supplemented with human serum albumin (20% v/v) (HAS, Vitrolife, 10064). The embryos were then transferred to the vitrification solution 1 (BM solution supplemented with DMSO (7.5% v/v) and ethylene glycol (7.5% v/v)) for 2–7 min. They were then transferred to the vitrification solution 2 (BM solution added DMSO (15% v/v), ethylene glycol (15% v/v), and sucrose (10% v/v)) for 30 seconds when shrank to 80%. They were then collected within 5–10 s in a minimal volume and transferred into a cryo device for immediate preservation in liquid nitrogen.

For thawing, the embryos were transferred from the cryo device to warming solution 1 (BM solution added 1 M sucrose) for 1 min and then to warming solution 2 (BM solution added 0.5 M sucrose) for 3 min. They were then transferred to basal medium for 5 min, washed using G2 solution and cultured 4 hours at 37 °C and 6% CO_2 (Allahbadia, et al., 2020; Korkmaz, et al., 2020).

Embryo selection and transfer

On the day of thawing, embryos were transferred. Embryos at both cleavage and blastocyst stages were assessed using a scoring system. Following thawing, the embryos were cultured for 4 to 6 hours at 37°C with 6% CO_2 before transplantation. Additionally, ultrasound scans were conducted to monitor endometrial thickening and receptivity in artificial cycles. The freeze-thawed embryos were transplanted on the third day of ovulation, specifically when the endometrium thickness was equal to or greater than 8 mm; hormone replacement treatment was administered for patients who did not experience natural ovulation. This included a daily dose of 2 to 4 mg of estradiol valerate starting from the third day of the menstrual cycle. Ultrasound-guided embryo transplantation was subsequently performed on days 18 to 19 (O'Shea, et al., 2016; Wang, et al., 2020).

Laser-assisted hatching procedure

The laser hatching procedure utilized a Nikon TE300 inverted microscope (Nikon, Tokyo, Japan) equipped with the Zona Infrared Laser Optical System (Hamilton-Thorne Research, Beverly, MA) employing a 1.48-mm infrared diode laser beam. Each embryo was placed on the microscope stage, and the laser was directed to the target area. The embryo was positioned to expose a Zona Pellucida (ZP) section to the laser beam. The laser was then activated and discharged multiple times, each lasting 0.5 ms, to create a single opening in the ZP, approximately 40 mm in size, called drilling. Zona thinning was made by putting a laser around the zona pellucida, estimated for 2 ms.

Data analysis

Group differences were tested using the t-test for numerical variables and the chi-square test for categorical variables using IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, N.Y., USA). A $p < 0.05$ was considered significant.

RESULT

The basic parameters evaluated using both methods are shown in Table 1. There are no significant differences between the LAH and no-LAH groups regarding average age, infertility duration, infertility type, and etiology of infertility (p>0.05). In the same line, the blastocyst (0.76 ± 0.87; 1.25 ± 1.08) compared with cleavage (0.72 ± 0.84; 0.67 ± 0.98) (p< 0.534 and p< 0.032; Table 1).

Table 1. Characteristic patients used LAH and no LAH.

Characteristics	LAH (-)	LAH(+)	p-values
Total patients	25	116	
Woman's age (year, mean ± sd)	34 ± 4.08	33.01 ± 4.78	0.360[b]
Man's age (year, mean ± sd)	38.7 ± 7.6	35.3 ± 5.46	0.069[c]
Duration of primary Infertility (year, mean ± sd)	5.15 ± 2.8	5.51 ± 3.5	0.961[c]
Duration of secondary infertility (year, mean ± sd)	11 ± NA	6.4 ± 4.50	0.380[c]
Diagnosis of infertility (n,%)			
Female factors	16 (64)	67 (68.3)	0.565
Male factors	23 (92)	114 (98.3)	0.145[d]
Unexplained infertility	2 (8)	9 (7.8)	1.000[d]
Mixed factors	16 (64)	67 (57.8)	0.565
Embryo transfer			
Cleavage transfer n=69 (mean ± sd)	0.72 ± 0.84	0.67 ± 0.98	0.534[c]
Blastocyst transfer n=72 (mean ± sd)	0.76 ± 0.87	1.25 ± 1.08	0.032[c]

Legends: [a]chi square test; [b] independent t-test; [c]Mann Whitney; [d]Fisher

Table 2. Outcome characteristics with LAH and no LAH.

Characteristics	LAH(-)	LAH(+)	p-values
Total patients	25	116	
Miscarriage (n,%)	1 (4)	8 (6.9)	1.000[d]
Failed (n,%)	18 (72)	69 (59.5)	0.243
Live birth	3 (12)	25 (21.6)	0.408[d]
BO (Blighted Ovum)	2 (8)	1 (0.9)	0.081[d]
Multiple pregnancies	1 (4)	7 (6)	1.000[d]
Biochemical	0 (0)	6 (5.2)	0.591[d]
Clinical pregnancy	1 (4)	15 (12.9)	0.305[d]

Legends: [a]chi square test; [b]independent t-test; [c]Mann Whitney; [d]Fisher

The comparison of patient outcome characteristics in Table 2 reveals no significant difference, even though clinically, the proportions of live births, multiple pregnancies, biochemical pregnancies, and ongoing pregnancies in the LAH group are not exceptions to the outcomes of failures and miscarriages.

Table 3. Pregnancy outcome characteristics with LAH and no LAH.

Characteristic	LAH(-) (25)	LAH(+) (116)	p-value
Failed (n,%)	18 (72%)	69 (59,5%)	0,243[a]
Success (n,%)	7 (28%)	47 (40,5%)	

Legends: [a]chi square test; [b] independent t-test; [c]Mann Whitney; [d]Fisher

The comparison of patient outcome characteristics in Table 3 shows no significant difference (p=0.243), even though clinically, the proportion of successful outcomes (live birth, multiple pregnancy, biochemical, and ongoing) in LAH (40.5%) is more significant than in no LAH (28%) when compared to failed outcomes.

Table 4. Outcome characteristics with total single hole dan 1/4 jole.

Characteristics	Drilling	Thinning	p-values
Total patients	84	32	
Miscarriage (n,%)	7 (8.3)	1 (3.1)	0.442[d]
Failed (n,%)	53 (63.1)	16 (50)	0.199
Live birth	16 (19)	9 (28.1)	0.288
BO (Blighted Ovum)	0 (0)	1 (3.1)	0.276[d]
Multiple pregnancies	5 (6)	2 (6.3)	1.000[d]
Biochemical	3 (3.6)	3 (9.4)	0.345[d]
Clinical pregnancy	13 (15.5)	2 (6.3)	0.231[d]

Legends: [a]chi square test; [b] independent t-test; [c]Mann Whitney; [d]Fisher

The comparison of patient outcome characteristics in Table 4 shows no significant difference, even though clinically, the proportion of live births, multiple pregnancies, and ongoing outcomes in drilling>thinning is not exempted in both failed and abortion outcomes.

DISCUSSION

The zona pellucida is a layer of glycoproteins that envelop the oocyte, comprising outer and inner layers. The outer layer is characterized by being thick and easily dissolved, while the inner layer is more resistant to dissolution. Its primary function is to prevent polyspermy and the fertilization of the oocyte by sperm without an intact acrosome. Implantation, the initial process involving the embryo's contact with the endometrium in the uterus, is termed hatching in its earlier stages. As the embryo reaches the blastocyst stage, it breaks free from the zona pellucida and initiates hatching. Inadequate blastocyst hatching may result in implantation failure during in vitro fertilization (IVF) (Lu, et al., 2019; Liu, et al., 2020).

Our findings revealed that the group undergoing LAH (laser-assisted hatching) exhibited a higher biochemical pregnancy rate compared to those who received no LAH treatment. Still, there is no significant correlation between the two groups. This aligns with research conducted by Endo, et al., (2021) indicating a significantly greater rate of complete hatching in the AH (assisted hatching) treatment group compared to the untreated control group. The use of LAH remains a subject of debate and controversy in the medical literature. According to the most recent national summary of AH provided by Hall, et al., (2017), there was a significant increase in AH utilization in the USA, rising from 25,725 to 35,518 procedures between 2000 and 2010. This upward trend might be associated with guidelines published in 2008 by the American Society for Reproductive Medicine (ASRM), suggesting the potential clinical usefulness of AH for patients with a poor prognosis (Liu, et al., 2020). The laser-assisted system offers notable advantages over chemical, mechanical, or enzymatic methods. Firstly, LAH allows for a touch-free, precisely targeted delivery of laser light to the desired location with minimal embryo absorption. Secondly, laser technology in human reproductive medicine is cost-effective and easily adaptable to inverted microscopes. The American Society for Reproductive

Medicine Practice Committee, (2022) reported significant clinical outcomes on FET using LAH. In contrast, Lacey, et al., (2021) indicated that ZP digestion by enzymatic methods was unrelated to any benefit of FET outcome. These differences could be due to the difference in the type of LAH or cryopreserved conditions such as cryoprotectant, stage of cryopreserved embryo, or storage duration.

Inadequate hatching can be influenced by the embryos from infertile female patients aged 38 years and above, leading to a notable decline in developmental performance and a significant reduction in fertility potential. Similar to the previous study, it resulted in a decrease in the pregnancy rate. Recent research suggests that this decline might be attributed to uncharacterized zona hardening in embryos due to advanced maternal age, impacting the quality of embryos with poor morphology. This zona hardening may hinder the blastocyst from breaking free from the ZP. Although there is no molecular evidence indicating prematurely cleaved ZP2 or other molecular markers associated with zona hardening in these embryos, other studies suggest that using LAH was similar between treated and untreated groups (18.36% compared to 11.36% in non-hatched embryos) within this specific group of patients (Nagy, et al., 2019).

The embryo's quality is another significant factor that could disrupt hatching. Various intrinsic elements, such as basal FSH levels and diagnoses, the hormonal changes experienced by maturing oocytes during ovarian stimulation, or the controlled conditions within the in vitro culture, can create obstacles for successful hatching. The artificial environment of in vitro culture might negatively impact the quantity or quality of zona lysin, a substance produced by the trophectoderm crucial for blastocyst hatching. There could also exist inherent variations in lysin secretion, independent of assisted reproduction techniques, contributing to the incapacity of certain blastocysts to hatch effectively (Tannus, et al., 2019; Curfs, et al., 2023). Embryo fragmentation stands as a common abnormality in human embryos. This detrimental influence can be attributed to various mechanisms, including disrupted mitotic spindles, reduced cell count, diminished cell size, partial or complete loss of regulatory proteins, disturbance in cell communication and signaling, reduced number of mitochondria, irregular mitochondrial distribution, decreased ATP content, failure in standard compaction and blastulation processes, discrepancies in cell allocation, and difficulties in hatching (Cecchele, et al., 2022).

Sagoskin and colleagues conducted a prospective, randomized, controlled trial. They found that assisted hatching (AH) did not enhance clinical pregnancy outcomes in patients with a good prognosis when only transferring good-quality embryos (Tannus, et al., 2019; Curfs, et al., 2023). The assisted hatching at the cleavage stage might expose the embryo to free radicals, toxins, and immune cells, leading to potential DNA damage and hindering its development to the blastocyst stage in the uterus. There is a possibility that during the compaction process, the loosely connected cells in 2 or 3-day-old embryos could be trapped or lost through the artificially created holes in the zona pellucida (ZP). Conversely, blastocyst-stage embryos consist of larger, more tightly connected groups of cells, potentially making them more resilient to localized damage caused by AH than embryos at the 4 to 8-cell stage. Repeated manipulation of the same embryo, first through intracytoplasmic sperm injection (ICSI) and later through AH, could be another mechanism negatively impacting the implantation potential of the embryo. Previous research indicated that this dual manipulation resulted in a lower clinical pregnancy rate when compared to either a single manipulation or no manipulation (Zhang, et al., 2022).

Recent research findings indicated that miscarriage rates remained comparable, whether using fresh embryos or those that had undergone cryopreservation and subsequent thawing for transfer. However, variations in clinical pregnancy and live birth rates were attributed to differences in implantation rather than pregnancy loss (Roeca, et al., 2020; Insogna, et al., 2021).

Strength and limitations

Laser zona-thinning increases the implantation rate, presumably higher in biochemical and clinical pregnancy. However, the primary outcome measure showed statistically insignificant findings between LAH and non-LAH. The methodology of this study appears to reduce bias by choosing a FET embryo based on cryopreserved condition, embryo quality, and precise manipulation using LAH that allows non-contact manipulation and avoidance of any injury to the embryo that causes an increased number of biochemical, clinical, multiple pregnancies, live birth rates. Although these differences were not statistically significant, in studies in a larger population, specific methods for non-LAH are needed to obtain a more robust and reliable evaluation of the superiority of the LAH method. The limitation of this study was a small number of samples with low quantities.

CONCLUSION

Laser-assisted hatching does not appear to increase the pregnancy rate in this study. However, the methodology reduces bias in this study by considering the method for selecting FET embryos based on the cryopreserved condition, embryo quality, and precise LAH.

Acknowledgment
The authors would like to thank all Bocah Indonesia Fertility Center staff in Tangerang, Indonesia, for their support.

Conflict of Interest
The authors have nothing to disclose.

Ethic consideration
The study has been approved by the Ethics Committee of Primaya Hospital in Tangerang City on 26-03-2022.

Funding Disclosure
None

Author Contribution
VL contributes to the conceptual and design, data analysis and interpretation, article drafting, and data curation. AR contributes to the conceptual and design, data analysis and interpretation, and article drafting. TD contributes to data analysis, interpretation, and critical revision of the article for important intellectual content. MDW contributes to data analysis and interpretation, critical revision of the article for important intellectual content, and final approval of the article.

REFERENCES

Allahbadia, G. N., Ata, B., Lindheim, S. R., et al. 2020. Textbook of assisted reproduction. Edited by G. N. Allahbadia et al. Singapore: Springer Singapore. doi: 10.1007/978-981-15-2377-9.

Blank, C., DeCroo, I., Weyers, B., et al. 2020. Improvement instead of stability in embryo quality between day 3-5: A possible extra predictor for blastocyst selection. European Journal of Obstetrics & Gynecology and Reproductive Biology, 253: 198–205. doi: 10.1016/j.ejogrb.2020.08.027.

Cecchele, A., Cermisoni, G. C., Giacomini, E., et al. 2022. Cellular and molecular nature of fragmentation of human embryos. International Journal of Molecular Sciences, 23(3): 1349. doi: 10.3390/ijms23031349.

Curfs, M. H. J. M., Cohlen, B. J., Slappendel, E. J., et al. 2023. A multicentre double-blinded randomized controlled trial on the efficacy of laser-assisted hatching in patients with repeated implantation failure undergoing IVF or ICSI. Human Reproduction, 38(10): 1952–1960. doi: 10.1093/humrep/dead173.

Endo, Y., Mitsuhata, S., Hayashi, M., et al. 2021. Laser-assisted hatching on clinical and neonatal outcomes in patients undergoing single vitrified Blastocyst transfer: A propensity score–matched study. Reproductive Medicine and Biology, 20(2),: 182–189. doi: 10.1002/rmb2.12366.

Gerri, C., Menchero, S., Mahadevaiah, S. K., et al. 2020. Human embryogenesis: A comparative perspective. Annual Review of Cell and Developmental Biology, 36(1): 411–440. doi: 10.1146/annurev-cellbio-022020-024900.

Hall, M. J., Schwartzman, A., Zhang, J., et al. 2017. Ambulatory surgery data from hospitals and ambulatory surgery centers: United States, 2010. National Health Statistics Reports, (102): 1014. Available at: https://www.cdc.gov/nchs/data/nhsr/nhsr102.pdf.

Hammadeh, M. E., Fischer-Hammadeh, C., Ali, K. R. 2011. Assisted hatching in assisted reproduction: A state of the art. Journal of Assisted Reproduction and Genetics, 28(2): 119–128. doi: 10.1007/s10815-010-9495-3.

IBM Corp. 2019. IBM SPSS statistics for Windows, version 26.0. Armonk, NY: IBM Corp. Available

at: https://www.ibm.com/support/pages/downloading-ibm-spss-statistics-26.
Insogna, I. G., Lanes, A., Lee, M. S., et al. 2021. Association of fresh embryo transfers compared with cryopreserved-thawed embryo transfers with live birth rate among women undergoing assisted reproduction using freshly retrieved donor oocytes. JAMA, 325(2): 156. doi: 10.1001/jama.2020.23718.
Jeong, J. E., Joo, B. S., Kim, C. W., et al. 2018. Effects of three-area laser-assisted zona thinning in 8-cell human embryos on pregnancy outcomes in vitro fertilization. Clinical and Experimental Reproductive Medicine, 45(1): 25–30. doi: 10.5653/cerm.2018.45.1.25.
Korkmaz, C., Gül Yıldız, Ü., Fidan, U., et al. 2020. Investigation of transfer results of human embryos that were vitrified and thawed at the cleavage, morula and blastocyst stages. Zygote, 28(3): 191–195. doi: 10.1017/S0967199419000777.
Lacey, L., Hassan, S., Franik, S., et al. 2021. Assisted hatching on assisted conception (in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI)). Cochrane Database of Systematic Reviews, 2021(4). doi: 10.1002/14651858.CD001894.pub6.
Le, M. T., Nguyen, T. T. A., Nguyen, T. T. T., et al. 2018. Thinning and drilling laser-assisted hatching in thawed embryo transfer: A randomized controlled trial. Clinical and Experimental Reproductive Medicine, 45(3): 129–134. doi: 10.5653/cerm.2018.45.3.129.
Liu, C., Su, K., Shang, W., et al. 2020. Higher implantation and live birth rates with laser zona pellucida breaching than thinning in single frozen-thawed blastocyst transfer. Lasers in Medical Science, 35(6): 1349–1355. doi: 10.1007/s10103-019-02946-7.
Lu, X., Liu, Y., Cao, X., et al. 2019. Laser-assisted hatching and clinical outcomes in frozen-thawed cleavage-embryo transfers of patients with previous repeated failure. Lasers in Medical Science, 34(6): 1137–1145. doi: 10.1007/s10103-018-02702-3.
Maheshwari, A., Bhattacharya, S., Bowler, U., et al. 2019. Study protocol: E-freeze - freezing of embryos in assisted conception: A randomised controlled trial evaluating the clinical and cost effectiveness of a policy of freezing embryos followed by thawed frozen embryo transfer compared with a policy of fresh. Reproductive Health, 16(1): 81. doi: 10.1186/s12978-019-0737-2.
Nagy, Z. P., Varghese, A. C., Agarwal, A. 2019. In vitro fertilization. Cham: Springer International Publishing. doi: 10.1007/978-3-319-43011-9.
Nieschlag, E., Behre, H. M., Kliesch, S., et al. 2023. Andrology: Male reproductive health and dysfunction. Springer Nature. Available at: https://books.google.co.id/books/about/Andrology.html?id=UJ7fEAAAQBAJ&redir_esc=y.
O'Shea, L. C., Hughes, C., Kirkham, C., et al. 2016. The impact of blastomere survival rates on developmental competence of cryo-thawed Day 2 embryos. European Journal of Obstetrics & Gynecology and Reproductive Biology, 197: 98–102. doi: 10.1016/j.ejogrb.2015.11.040.
Practice Committee of the American Society for Reproductive Medicine. 2022. The role of assisted hatching in in vitro fertilization: a guideline. Fertility and Sterility, 117(6): 1177–1182. doi: 10.1016/j.fertnstert.2022.02.020.
Roeca, C., Johnson, R. L., Truong, T., et al. 2020. Birth outcomes are superior after transfer of fresh versus frozen embryos for donor oocyte recipients. Human Reproduction, 35(12): 2850–2859. doi: 10.1093/humrep/deaa245.
Siristatidis, C., Stavros, S., Dafopoulos, K., et al. 2022. A randomized controlled trial on the efficacy and safety of low-dose hCG in a short protocol with GnRH agonist and ovarian stimulation with recombinant FSH (rFSH) during the follicular phase in infertile women undergoing ART. Reproductive Sciences, 29(2): 497–505. doi: 10.1007/s43032-021-00683-3.
Stigliani, S., Massarotti, C., Bovis, F., et al. 2021. Pronuclear score improves prediction of embryo implantation success in ICSI cycles. BMC Pregnancy and Childbirth, 21(1): 361. doi: 10.1186/s12884-021-03820-7.
Tannus, S., Cohen, Y., Henderson, S., et al. 2019. The effect of assisted hatching on live birth rate following fresh embryo transfer in advanced maternal age. Reproductive Sciences, 26(6): 806–811. doi: 10.1177/1933719118799192.
Wang, Y., Liu, W. J., Fan, L., et al. 2020. The impacts of the number of prefreeze and postthaw blastomeres on embryo implantation potential. Medicine, 99(13): e19591. doi: 10.1097/MD.0000000000019591.
Wang, Y., Chen, C., Liang, J., et al. 2022. A comparison of the clinical effects of thinning and drilling on laser-assisted hatching. Lasers in Medical Science, 37(1): 1–9. doi: 10.1007/s10103-020-03230-9.

Wei, C., Xiang, S., Liu, D., et al. 2023. Laser-assisted hatching improves pregnancy outcomes in frozen-thawed embryo transfer cycles of cleavage-stage embryos: A large retrospective cohort study with propensity score matching. Journal of Assisted Reproduction and Genetics, 40(2): 417–427. doi: 10.1007/s10815-022-02711-w.

Zhang, L., Zhou, Y., Wu, Y. J., et al. 2022. Thinning or opening: A randomized sibling-embryo pilot trial on the efficacy of two laser-assisted hatching modes during the extended culture of highly fragmented cleavage embryos. Frontiers in Endocrinology, 13. doi: 10.3389/fendo.2022.927834.

SYSTEMATIC REVIEW

Features of the Clinical Manifestations of Autoimmune Optic Neuropathy in Multiple Sclerosis on Corticosteroid Therapy

Dalia Sharliz Raihana[1], Lukiasari Agustini[2*], Deasy Fetarayani[3], Agus Ariyanto[4]
[1]Faculty of Medicine, University Airlangga, Surabaya, Indonesia.
[2]Department Opthalmology, Faculty of Medicine, University Airlangga, Surabaya, Indonesia.
[3]Department of Internal Medicine, Faculty of Medicine, University Airlangga, Surabaya, Indonesia; Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.
[4]Department of Opthalmologist, Imanuddin General Hospital, West Kotawaringin, Indonesia.

Article Info
Article history:
Received 02-01-2024
Revised 05-06-2024
Accepted 21-06-2024
Published 01-07-2024

Keywords:
Optic neuropathy
Quality
Multiple sclerosis (MS)
Optic neuritis treatment trial (ONTT)

***Corresponding author**:
Lukiasari Agustini
lukiasari.agustini@fk.unair.ac.id

ABSTRACT

Background: The clinical picture of autoimmune optic neuropathy, known as neuritis, is strongly influenced by the unique structure of the human anterior visual pathway. The central nervous system autoimmune is related to multiple sclerosis (MS). Optic neuropathy is an injury that frequently results in acute inflammatory damage. **Objective**: This study aimed to determine the clinical manifestations of autoimmune optic neuropathy in multiple sclerosis and identify diseases with appropriate corticosteroid therapy using systematic review methods. **Material and Method**: This study used a systematic review method to analyze topic-related kinds of literature on Scopus, PubMed, and Google Scholar databases. The literature screening process was carried out based on the PRISMA 2020 guidelines. **Result**: Regarding the post-treatment recurrence rate of optic neuritis, the use of intravenous corticosteroids alone and intravenous corticosteroid followed by oral administration may clinically reduce the incidence of recurrence in the patients compared those receiving placebo and oral administration. This suggests that intravenous corticosteroid followed by oral corticosteroid treatment is effective in helping to reduce the incidence of recurring optic neuritis. **Conclusion**: Intravenous corticosteroid treatment followed by oral administration may clinically reduce the incidence of post-treatment recurrence of optic neuritis in multiple sclerosis (MS) patients.

How to cite:
Raihana, D.S., Agustini, L., Fetarayani, D. 2024. Features of the Clinical Manifestations of Autoimmune Optic Neuropathy in Multiple Sclerosis on Corticosteroid Therapy. Majalah Biomorfologi-Biomorphology Journal, 34(2): 123-133.

Majalah Biomorfologi (Biomorphology Journal) p.ISSN:0215-8833, e.ISSN: 2716-0920
doi: 10.20473/mbiom.v34i1.2024.123-133

Highlights

1. Intravenous corticosteroid treatment followed by oral clinical treatment can reduce the incidence of post-treatment optic neuritis recurrence.
2. Visual acquisition increases at one-month post-treatment.

BACKGROUND

The clinical picture of autoimmune optic neuropathy, also known as neuritis, is strongly influenced by the unique structure of the human anterior visual pathway. This central nervous system autoimmune disease is related to multiple sclerosis (MS) (Kawachi, 2017). Optic neuropathy is an injury that often causes acute inflammatory damage with the typical diagnoses of vision loss, colour vision impairment, decreased brightness perception, afferent pupillary deficiency, nerve fibre layer disruption, optic nerve pallor, or optic nerve swelling (Ghadiali & Odel, 2019). Optic neuritis has a strong connection with MS, which is an autoimmune disease that attacks certain individuals. Optic neuropathy associated with MS can manifest in two forms: opticospinal multiple sclerosis (OS-MS), which is accompanied by cerebral lesions, and neuromyelitis optica (NMO), which is not accompanied by cerebral lesions. These two forms are still not specific entities. Some associated eye symptoms include pain, especially pain exacerbated by eye movement, diplopia, and positive visual phenomena (Pineles & Balcer, 2019). According to Bennett's (2019) study results, optic nerve inflammation may result from various causes, such as autoimmunity.

Optic neuritis is a type of inflammatory optic neuropathy that is often associated with autoimmune neurological diseases, such as MS, myelin-oligodendrocyte glycoprotein antibody-related diseases, and optic neuromyelitis spectrum disorders (Bennett, et al., 2023). When suspicion of clinically isolated syndrome (CIS) or MS arises, a brain and spinal cord scan should be performed. In addition to providing information regarding the distribution of the lesion in the space, an MRI of the spinal cord is also functional for differential diagnosis when there is uncertainty regarding the nature of the brain lesion. Spinal cord MRI can reveal disease activity without symptoms and predict disease evolution (Tomassini, et al., 2020). The exact cause of MS is not yet known. Still, several journals show evidence that part of the immunopathogenesis of MS is derived from demyelinating antibody responses resulting in inflammation of the central nervous (CNS) with varying clinical presentations leading to the contribution of autoimmune manifestations. MS remains elusive and is thought to involve genetic and environmental factors with no definitive treatment. The prevalence of this disease has a peak between the ages of 20-40 years, but it has been proven also to affect children and elderly people over the age of 60 years. MS affects women about twice as often as men (Bennett, 2019). According to the Optic Neuritis Treatment Trial (ONTT), initial therapy for patients with demyelination high-dose corticosteroids intravenously or orally, which is standard practice for patients with optic neuropathy to reduce the severity of attacks and prevent permanent damage (Morrow, et al., 2018). Corticosteroids are a drug that is often used and is relatively cheap to reduce inflammation (Bennett, et al., 2023).

This study aimed to provide a deeper understanding and overall facts regarding appropriate corticosteroid therapy by differentiating the route of administration for MS patients. The findings will be summarized in detail to cover a comprehensive level of knowledge and understanding. Further case studies of the impact of corticosteroid treatment therapy on patients will be presented by providing relevant facts.

OBJECTIVE

This systematic review aimed to determine the clinical manifestations of autoimmune optic neuropathy in multiple sclerosis and identify diseases with appropriate corticosteroid therapy.

MATERIAL AND METHOD

This study used a systematic review method to analyze topic-related literature in the Scopus, PubMed, and Google Scholar databases. This systematic review registration number in PROSPERO is CRD42023491771. Retrieving literature to determine data quality used the main version of the 2020 PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). Before entering the screening stage, researchers eliminated studies that were not relevant because the title did not match the topic. Duplicate studies were from the same journal, only manually taken from one database. Manual searches were carried out to reduce the bias of inappropriate keywords, making it easier to continue

screening the articles further. Data screening was done on the condition that it met the inclusion criteria, and the data were excluded if it did not match the research topic.

The PICO (Population, Intervention, Comparison, Outcomes) approach was used in this study. The population was patients suffering from multiple sclerosis (MS) with optic neuropathy. The intervention was intravenous and oral corticosteroids, while the comparison was not applied. The outcome included the clinical features (VEP or Visual Evoked Potential and VA or Visual Acuity)

The included research articles must be accessible in full-text literature that explains the manifestations of autoimmune optic neuropathy in multiple sclerosis (MS) and research that uses case-control or cross-sectional study methods. The articles that could not be included were those involving patients who had MS without symptoms of optic neuropathy, studies that used systematic review methods, and those that did not discuss MS.

RESULT

The PRISMA guidelines were used for the procedure and evaluation of the systematic review, as shown in Figure 1. On December 11, 2023, literature was carried out on the Scopus, PubMed, and Google Scholar databases. The following is the PRISMA flowchart made in this study.

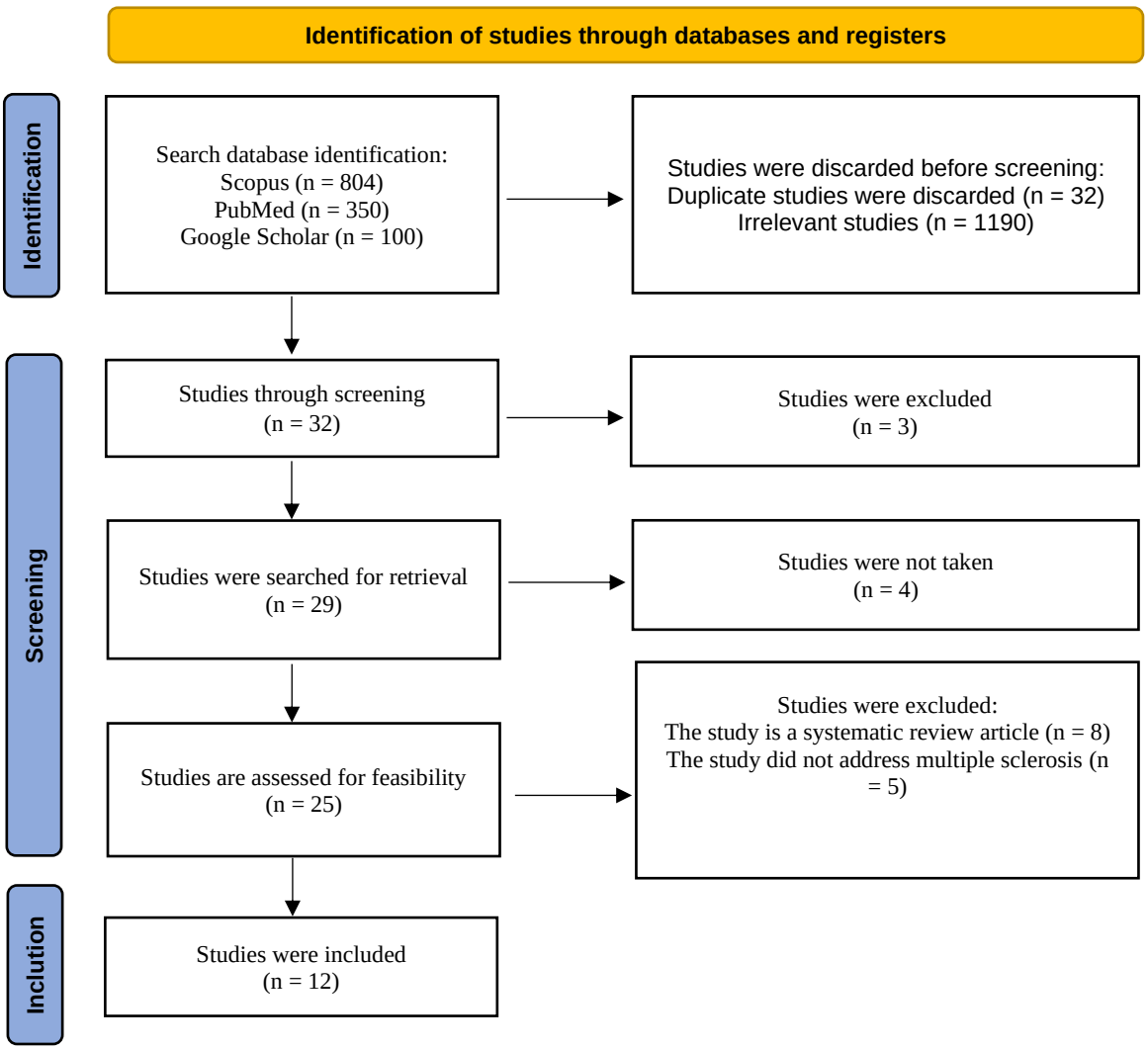

Figure 1. PRISMA systematic review diagram 2020 category.
"new systematic reviews which included searches of databases and registers only"
(Page, et al., 2021).

All of the included articles underwent quality assessment using critical appraisal tools, as shown in Tables 1, 2, 3, and 4.

Table 1. Data extraction results from reviewed journals.

No.	Reference	Amount of samples	Intervention		Results			Information
			Orally	Intravenous	Variable	Before	After	
1.	Smith, et al. (1986)	Eight patients	-	1000 mg Methylprednisolone 1x1 for three days.	P100 latency of visual-evoked potential & visual acuity for one month.	132 ± 3.3 MS & 6/18	127 ± 5.8 MS & 6/6	Shortening mean P100 latency of visual-evoked potential and repaired visual acuity. The shortening latency value shows the process of remyelination from neurons.
2.	Kapoor et al., (1998)	25 patients	-	1000 mg Methylprednisolone 1x1 for three days.	P100 latency of visual-evoked potential & visual acuity for six months.	130.5 ± 19.0 MS & 0.63	122.7 ± 15.0 MS & 0.68	Shortening mean P100 latency of visual-evoked potential and repaired visual acuity. The shortening latency value shows the process of remyelination from neurons.
3.	Morrow, et al., (2018)	23 patients	Prednisone 1250 mg	1000 mg Methylprednisolone 1x1 for three days.	P100 latency of visual-evoked potential & visual acuity for six months.	181.9 ± 53.6 MS & 20/100	119.0 ± 16.5 MS & 20/160	The decline means P100 latency of visual-evoked potential & a decrease in visual acuity.
4.	Menon, et al., (2007)	11 patients	-	200 mg Dexamethasone 1x 1 for 3 days.	LOGMAR visual acuity.	1.10 ± 0.52	0.28 ± 0.33	After three months, the visual acuity was repaired.

5.	Naumovska, et al., (2018)	28 Patients	Methylprednisolone 500 mg	500 mg Methylprednisolone.	LOGMAR visual acuity.	Orally: 0.30 IV: 0.35	Orally: 0.05 IV: 0.05	There is fast repair of visual acuity.
6.	de Lott, et al., (2020)	335 patients	-	500 mg Methylprednisolone.	LOGMAR Visual Acuity	0.305	0.084	It happens to repair visual acuity faster.
7.	Beck, et al., (1992)	15 6 patients orally, 151 patients intravenous	Prednisonene 1 mg/kg ww/day for 14 days	1000 mg Methylprednisolone intravenous 1x1 for three days followed with prednisolone orally 1 mg/kg ww/day for 1 1 day.	Percentage of visual acuity after six months.	58.7%	Intravenous:60.9% PO: 54.5%	There is enhanced sensitivity contrast, color vision, visual acuity, and visual fields, which are faster in the intravenous group.
8.	Goodwin, (1996)	438 patients	Prednisonene 1 mg/kg ww/day for 14 days.	1000 mg Methylprednisolone intravenous 1x1 for three days followed by prednisolone orally 1 mg/kg ww/day for 11 days.	Percentage of patients who Still have visual function six months post-maintenance.	5.3%	IV: 6.0% Orally: 7.1%	Enhancement of visual functionality: One week first, IV is faster than orally.
9.	Al-Eajailat & Al-MadaniSenior, (2014)	50 patients placebo, 50IV patients, 50 patients P.O	Prednisonene 1 mg/kg ww/day for 14 days.	1000 mg Methylprednisolone intravenous 1x1 for three days followed by prednisolone orally 1 mg/kg ww/day for 11 days.	The amount patients who experience enhanced visual acuity is a minimum of 50% after four weeks	IV:84 % Orally: 86%	IV: 94% Orally: 94%	Shortening means P100 latency of visual-evoked potential and improve-ments in visual acquisition.
10.	Halilovic, et al., (2014)	Ten patients	-	1000 mg Prednisolone intravenous 1x3 days followed by prednisolone 1 mg/kg/d for eight days.	Visual acuity	0.2 ± 0.1	0.8 ± 0.2	The visual acquisition increased at one-month post-treatment.
11.	Dahanayake, et al., (2021)	29 patients	-	1000 mg Methylprednisolone intravenous 1x3 days followed with prednisone ne 1 mg/kg ww/day for 11 days.	Decline P100 latency of visual-evoked potential	131.85 ± 10.85 MS	110.7 ± 12.5 MS	Decline P100 latency from visual-evoked potential to average value one month after treatment.

12.	Sellebjerg, et al., (1999)	60 patients	500 mg oral Methylprednisolone 1x1 for five days. Later dose tapering for ten days.	-	Percentage of patients who reach visual acuity normal after 8 Sunday	Placebo :43%	Orally:50%	There isn't any significant difference in visual acuity between the oral and placebo groups.

Table 2. The effect of corticosteroid administration on VEP.

No.	Reference	Intervention		Variable	Results	Information
		Orally	Intravenous			
1.	Smith, et al. (1986)	-	1000 mg Methylprednisolone 1x3 in 3 days.	P100 latency of visual-evoked potential & visual acuity for one month.	Before: 132 ±3.3 MS & 6/18. After: 127 ± 5.8 MS & 6/6.	Shortening mean P100 latency of visual-evoked potential and repaired visual equity. The shortening latency value shows that remyelination from neurons has occurred.
2.	Kapoor et al., (1998)	-	1000 mg Methylprednisolone 3x1 day.	The observed variables are P100 latency of visual-evoked potential & visual acuity for one month.	Before: 130.5 ± 19.0 MS & 0.63 After: 122.7 ± 15.0 MS & 0.68	Shortening means P100 latency of visual-evoked potential and repaired visual acuity. The shortening latency value shows that remyelination process from the nerve cell has occurred.
3.	Dahanayake, et al., (2021)	-	1000 mg intravenous Methylprednisolone 1x1 for three days followed with prednisonene 1 mg/kg ww/day for 11 days.	Declined P100 latency of visual-evoked potential	Before: 131.85 ± 10.85 MS After: 110.7 ± 12.5 MS	Declined P100 latency of visual-evoked potential to normal value one month after treatment, indicating remyelination.
4.	Morrow, et al., (2018)	1250 mg Prednisone	-	P100 latency of visual-evoked potential & visual acuity for six months.	Before : 181.9 ± 53.6 MS & 20/100 After : 9.0 ±16.5 MS & 20/160	The declined means P100 latency of visual-evoked potential, which indicates the remyelination process.

Table 3. The effect of corticosteroid administration on visual acuity.

No.	Reference	Intervention		Variable	Results	Information
		Orally	Intravenous			
1.	Beck, et al., (1992)	1 mg/kg oral Prednisolone ww/day for 11 days.	-	Percentage of patients who have visual acuity after six months.	Intravenous= 60.9 % Orally=54.5%	There is enhanced sensitivity contrast, color vision, visual acuity, and visual fields, which are faster than the oral group.
2.	Goodwin, (1996)	1 mg/kg Prednisone ww/day for 14 days.	-	Percentage of patients who still own visual functions, the bad ones six months post-care.	Intravenous 6% per oral 7.1%.	There isn't any significant difference between IV and placebo at six months; however, enhanced visual function in 1 first week is faster than oral.
3.	Al-Eajailat & Al-MadaniSenior, (2014)	1 mg/kg/day oral prednisolone for 14 days	-	The amount of patients who experience enhanced visual acuity minimum 50% after four weeks compared to placebo.	Intravenous= 47 person Orally= 47 person	There were no differences between the treatment group and the control group.
4.	Halilovic, et al., (2014)	-	-	Visual acuity	Before: 0.2 ± 0.1 After: 0.8 ± 0.2.	The visual acquisition increased one month post-treatment
5.	Sellebjerg, et al., (1999)	-	-	The percentage of patients who reach visual acuity is expected after 8 weeks.	Oral: 50% P placebo 43%.	There isn't any significant difference in visual acuity between oral and placebo groups.

Table 4. The effect of corticosteroid on LOGMAR visual acuity

No.	References	Intervention		Variables	Results	Information
		Orally	Intravenous			
1.	Menon, et al., (2007)	200 mg Dexamet hasone 1x3 days.	-	LOGMAR visual acuity	Before: 1.10 ± 0.52 After: 0.28 ± 0.33	It happens to repair visual acuity three months after treatment.
2.	Naumovska, et al., (2018)	500 mg Methylpre prednisolone	-	LOGMAR visual acuity	Before: Oral: 0.30 IV:0.35 After: Orally: 0.05 IV: 0.05	There is visual acuity improvement, faster compared to placebo.
3.	de Lott, et al., (2020)	-	500 mg Methylpr prednisolone	LOGMAR visual acuity	Before : 1.10 ± 0.52 After : 0.28 ± 0.33	After three months of repair, visual acuity indicating remyelination has occurred.

DISCUSSION

Multiple sclerosis is an autoimmune disease with a prevalence of 35.9 individuals per 100,000 population. Based on existing data, this number has continued to increase every year since 2013 (Walton, et al., 2020). Multiple sclerosis is a disease that can significantly reduce the patients' quality of life. One of the clinical signs of multiple sclerosis is a decrease in the quality of vision, so many studies are looking for the most appropriate way to improve the quality of vision among the multiple sclerosis patients. One treatment widely studied to overcome this problem is corticosteroid therapy (Ozdogar, et al., 2022).

Research conducted by Smith, et al., (1986), Kapoor, et al., (1998), and Morrow, et al., (2018) observed the effects of methylprednisolone corticosteroid treatment at a dose of 1000 mg given to patients once a day for three days through an intravenous route based on improved visual evoked potential (VEP) latency. These three studies found improvements in mean VEP latency after one month and six months of treatment, but these changes were insignificant. There is a possibility that the improvement in mean VEP may not be due to intravenous corticosteroid treatment because the group that did not receive treatment also experienced an improvement in mean VEP (Smith, et al., 1986).

Recent research by Menon, et al., (2007); Naumovska, et al., (2018); de Lott, et al., (2020) showed that 500 mg intravenous methylprednisolone or 200 mg dexamethasone improve visual acuity in symptoms of optic neuritis experienced by multiple sclerosis patients. These three studies, using methylprednisolone and dexamethasone, found a faster visual acuity increase compared to the placebo group. Still, the two groups had no significant difference in the final visual acuity value. Studies by Beck, et al., (1992), Cleary, et al., (1993), Goodwin, (1996), Menon, et al., (2007), Al-Eajailat & Al-MadaniSenior, (2014), Halilovic, et al., (2014), and Dahanayake, et al., (2021) observed the effect of treatment using 1000 mg intravenous methylprednisolone once a day for three days followed by 1 mg/kg oral prednisone once a day for 14 days on visual acuity in multiple sclerosis patients who experienced optic neuritis. These eight studies showed uniform results. The patients who received intravenous corticosteroid treatment followed by oral therapy experienced a faster increase in visual acuity compared to the group that received placebo treatment. However, no significant differences were found between the two groups in evaluating the final visual acuity scores six months or one year after treatment.

Then, the researchers Beck, et al., (1992), Cleary, et al., (1993), Goodwin, (1996), Gal, et al., (2012), and Al-Eajailat & Al-MadaniSenior, (2014) examined the effects of 1 mg/kg oral prednisolone treatment once a day for 14 days on visual acuity, visual function, visual fields, contrast sensitivity, and VEP latency. These three studies showed uniform results in that all parameters improved after treatment, but this did not increase significantly compared to patients who received treatment with a placebo. In addition, these three studies showed that patients who received oral corticosteroid treatment experienced a higher rate of optic neuritis recurrence than patients who received placebo treatment.

Researchers observed the effects resulting from 500 mg oral methylprednisolone treatment once a day for five days continued with tapering doses for ten days (Sellebjerg, et al., 1999; Naumovska, et al., 2018). Both studies showed uniform results, in which there were improvement in visual acuity, colour vision, and contrast sensitivity, between the group that received oral corticosteroid treatment and the group that only received placebo treatment. Still, there were no significant differences between the two groups. A Study using 1250 mg prednisone showed improved VEP six months after treatment (Morrow, et al., 2018).

In general, treatment of optic neuritis in multiple sclerosis patients using intravenous corticosteroids alone, intravenous followed by oral, or oral alone shows an increase in VEP. However, the differences experienced are not significant, and there is an increase in visual acuity. The increase in the average of both parameters is due to corticosteroid treatment, which reduces inflammation and edema that forms on the optic disc. Reducing inflammation and edema that forms on the optic disc can reduce demyelination and improve conduction in the demyelinated nerve area, which can help speed up the course of stimulus conduction (de Lott, et al., 2022). The non-significant difference in VEP latency but significant in visual acuity is probably because the VEP latency test is a test that has higher sensitivity and specificity than visual acuity so that a more important clinical change is needed to show a more statistically significant change in VEP latency values (Ismaiel, et al., 2020).

This suggests that in terms of improving visual function, corticosteroid treatment is effective in accelerating changes but does not improve the outcome of improving visual function (de Lott, et al., 2022). Regarding post-treatment optic neuritis recurrence rates, studies using intravenous corticosteroids alone and intravenous followed oral corticosteroid groups showed reduced incidence of recurrence in patients compared to the placebo and oral groups. The group using oral corticosteroids alone did not show a reduced incidence of optic neuritis recurrence in patients compared to the placebo group (Al-Eajailat & Al-MadaniSenior, 2014).

In ONTT, findings were shown in optic neuritis patients with oral and intravenous prednisolone. Based on the description above, it can be observed that optic neuritis treatment using corticosteroids with various routes can be considered based on multiple considerations. The first one is whether the patient needs immediate improvement in visual function. Patients needing to improve visual function quickly can consider treatment using corticosteroids (Gal, et al., 2012). The second one that can be considered is that in reducing the recurrence rate of optic neuritis, intravenous corticosteroid only or followed by the oral administration has advantages over oral treatment alone (Al-Eajailat & Al-MadaniSenior, 2014). This raises further considerations, that the higher toxicity of intravenous corticosteroid treatment and its adverse effects may occur as a result of the treatment. Some of the adverse effects reported to be experienced by multiple sclerosis patients with optic neuritis and receiving corticosteroid treatment include transient depression, acute pancreatitis, sleep disturbances, mood swings, stomach discomfort, facial flushing and weight gain (Gal, et al., 2012).

Strength and limitations

The strength of this review is that it has conducted a comprehensive analysis of relevant articles to demonstrate that administering intravenous corticosteroids followed by oral clinical therapy can effectively decrease the occurrence of recurrent optic neuritis after treatment. Additionally, it has been observed that visual acquisition improves one month after therapy. However, the limitation of this study is that it should have gathered more recent studies regarding the case.

CONCLUSION

Clinically, intravenous corticosteroid treatment, followed by oral administration, can accelerate visual function improvement in visual acuity and visual evoked potential (VEP) latency. Intravenous corticosteroid only and intravenous corticosteroid followed by oral treatment can also reduce the incidence of post-treatment optic neuritis recurrence.

Acknowledgment

The authors would like to thank the Medical Staff of the Department of Opthalmology, Faculty of Medicine, University of Airlangga, and the Department of Internal Medicine, Faculty of Medicine, University of Airlangga.

Conflict of Interest

All authors have no conflict of interest.

Funding Disclosure

None.

Author Contribution

DSR contributes to the conception and design, analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, provision of study materials or patients, administrative, technical, or logistic support, and collection and assembly of the data. LA and DF contribute to the final approval of the article and provision of study materials or patients.

REFERENCES

Al-Eajailat, S. M., Al-MadaniSenior, M. V. 2014. The role of magnetic resonance imaging and visual evoked potential in management of optic neuritis. Pan African Medical Journal, 17. doi:

10.11604/pamj.2014.17.54.2462.
Beck, R. W., Cleary, P. A., Anderson, M. M., et al. 1992. A randomized, controlled trial of corticosteroids in the treatment of acute optic neuritis. New England Journal of Medicine, 326(9): 581–588. doi: 10.1056/NEJM199202273260901.
Bennett, J. L. 2019. Optic neuritis. Continuum: Lifelong Learning in Neurology, 25(5): 1236–1264. doi: 10.1212/CON.0000000000000768.
Bennett, J. L., Costello, F., Chen, J. J., et al. 2023. Optic neuritis and autoimmune optic neuropathies: advances in diagnosis and treatment. The Lancet Neurology, 22(1): 89–100. doi: 10.1016/S1474-4422(22)00187-9.
Cleary, P. A., Beck, R. W., Anderson, M. M., et al. 1993. Design, methods, and conduct of the optic neuritis treatment trial. Controlled Clinical Trials, 14(2): 123–142. doi: 10.1016/0197-2456(93)90015-6.
Dahanayake, P., Beck, R. W., Anderson, M. M., et al. 2021. The effect of optic neuritis treatment trial (ONTT) combined corticosteroid regimen on pattern reversal visual evoked potentials: A prospective follow-up study. BMC Ophthalmology, 21(1): 324. doi: 10.1186/s12886-021-02086-z.
Gal, R. L., Vedula, S. S., Beck, R. 2012. Corticosteroids for treating optic neuritis. in Gal, R. L. (ed.) Cochrane Database of Systematic Reviews. Chichester, UK: John Wiley & Sons, Ltd. doi: 10.1002/14651858.CD001430.pub3.
Ghadiali, L. K., Odel, J. G. 2019. Optic neuropathy. in The Columbia Guide to Basic Elements of Eye Care. Cham: Springer International Publishing: 399–427. doi: 10.1007/978-3-030-10886-1_36.
Goodwin, J. 1996. High dosage corticosteroids in the treatment of optic neuritis and prophylaxis of multiple sclerosis. Clinical Immunotherapeutics, 6(5): 405–412. doi: 10.1007/BF03259359.
Halilovic, E. A., Alimanovic, I., Suljic, E., et al. 2014. Optic neuritis as first clinical manifestations the multiple sclerosis. Materia Sociomedica, 26(4): 246–248. doi: 10.5455/msm.2014.246-2481.
Ismaiel, A., Mohsen, A., Anwar, A. 2020. Comparative study between visual evoked potential and visual acuity, a field of vision, and fundus examination as a screening tool for early diagnosis of hydroxychloroquine and chloroquine retinal toxicity in rheumatic patients. Journal of Medicine in Scientific Research, 3(3): 161. doi: 10.4103/JMISR.JMISR_29_20.
Kapoor, R., Miller, D. H., Jones, S. J., et al. 1998. Effects of intravenous methylprednisolone on outcome in MRI-based prognostic subgroups in acute optic neuritis. Neurology, 50(1): 230–237. doi: 10.1212/WNL.50.1.230.
Kawachi, I. 2017. Clinical characteristics of autoimmune optic neuritis. Clinical and Experimental Neuroimmunology, 8(S1): 8–16. doi: 10.1111/cen3.12354.
de Lott, L. B., Burke, J. F., Andrews, C. A., et al. 2020. Association of individual-level factors with visual outcomes in optic neuritis. JAMA Network Open, 3(5): e204339. doi: 10.1001/jamanetworkopen.2020.4339.
de Lott, L. B., Bennett, J. L., Costello, F. 2022. The changing landscape of optic neuritis: A narrative review. Journal of Neurology, 269(1): 111–124. doi: 10.1007/s00415-020-10352-1.
Menon, V., Mehrotra, A., Saxena, R., et al. 2007. Comparative evaluation of megadose methylprednisolone with dexamethasone for treatment of primary typical optic neuritis. Indian Journal of Ophthalmology, 55(5): 355. doi: 10.4103/0301-4738.33821.
Morrow, S. A., Fraser, J. A., Day, C., et al. 2018. Effect of treating acute optic neuritis with bioequivalent oral vs intravenous corticosteroids. JAMA Neurology, 75(6): 690. doi: 10.1001/jamaneurol.2018.0024.
Naumovska, M., Sheikh, R., Bengtsson, B., et al. 2018. Visual outcome is similar in optic neuritis patients treated with oral and i.v. high-dose methylprednisolone: A retrospective study on 56 patients. BMC Neurology, 18(1): 160. doi: 10.1186/s12883-018-1165-6.
Ozdogar, A. T., Baba, C., Kahraman, T., et al. 2022. Effects and safety of exergaming in persons with multiple sclerosis during corticosteroid treatment: A pilot study. Multiple Sclerosis and Related Disorders, 63: 103823. doi: 10.1016/j.msard.2022.103823.
Page, M. J., McKenzie, J. E., Bossuyt, P. M., et al. 2021. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. BMJ: 71. doi: 10.1136/bmj.n71.
Pineles, S. L., Balcer, L. J. 2019. Visual loss. in Liu, Volpe, and Galetta's Neuro-Ophthalmology. Elsevier: 101–196. doi: 10.1016/B978-0-323-34044-1.00005-5.
Sellebjerg, F., Nielsen, H. S., Frederiksen, J. L., et al. 1999. A randomized, controlled trial of oral high-

dose methylprednisolone in acute optic neuritis. Neurology, 52(7): 1479–1479. doi: 10.1212/WNL.52.7.1479.

Smith, T., Zeeberg, I., Sjö, O. 1986. Evoked potentials in multiple sclerosis before and after high-dose methylprednisolone infusion. European Neurology, 25(1): 67–73. doi: 10.1159/000115989.

Tomassini, V., Sinclair, A., Sawlani, V., et al. 2020. Diagnosis and management of multiple sclerosis: MRI in clinical practice. Journal of Neurology, 267(10): 2917–2925. doi: 10.1007/s00415-020-09930-0.

Walton, C., King, R., Rechtman, L., et al. 2020. Rising prevalence of multiple sclerosis worldwide: Insights from the Atlas of MS, third edition. Multiple Sclerosis Journal, 26(14): 1816–1821. doi: 10.1177/1352458520970841.

SCOPING REVIEW

Osteoinductive Capacity of Platelet-Rich Fibrin *vs.* Biodentine for Mandible Fracture

Marda Agung Nugraha[1], Indra Mulyawan[2*], Ardian Jayakusuma Amran[3,4]
[1]Resident of Oral and Maxillofacial Surgery, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.
[2] Department of Oral and Maxillofacial Surgery, Faculty of Dental Medicine-Academic Dental Hospital, Universitas Airlangga, Surabaya, Indonesia.
[3]Doctoral Program of Dentistry, Faculty of Dentistry, Universitas Hasanuddin, Makassar, Indonesia.
[4] Department of Oral and Maxillofacial Surgery, Faculty of Dental Medicine, Universitas Muslim Indonesia, Makassar, Indonesia.

Article Info

Article history:
Received 07-08-2023
Revised 17-04-2024
Accepted 26-04-2024
Published 01-07-2024

Keywords:
Mandible fracture
Osteoinductive
Platelet Rich Fibrin
Biodentine
Human & health

***Corresponding author**:
Indra Mulyawan
indramulyawan@fkg.unair.ac.id

ABSTRACT

Background: Mandibular fracture is one of the most common fractures. The most common treatment for mandibular fractures is fixation. Therefore, xenogeneic agents such as platelet-rich fibrin (PRF) and Biodentine accelerate the reparative process. Biodentine is an interesting active ingredient that can induce bone regeneration. PRF and Biodentine can promote bone healing, but no literature discusses the differences between PRF and Biodentine osteoinduction mechanisms in treating mandibular fractures. **Objective**: This article aimed to compare the effect of osteoinductive PRF with Biodentine for mandibular fractures. **Material and Method**: The research was conducted as a scoping review by performing a thorough search of the PubMed, Scopus, ScienceDirect, Elsevier, and Google Scholar databases. The study was obtained based on literature studies in the form of journals and textbooks in the last ten years (2013-2023). **Result**: The osteoinductive effect and mechanism of Biodentine in enhancing bone repair are likely correlated with releasing biologically active ions from calcium silicate cement and stimulating gene expression Runx-2. PRF has an osteoinductive role, causing the mechanism of releasing growth factors such as PDGF, VEGF, TGF-β, and IGF that promote the osteogenic process. **Conclusion**: There was no significant difference in the osteoinduction effect of PRF and Biodentine because these materials have different mechanisms of action for bone repair.

How to cite:
Nugraha, M.A., Mulyawan, I., Jayakusuma, A. A. 2024. Osteoinductive Capacity of Platelet-Rich Fibrin vs Biodentine for Mandible Fracture. Majalah Biomorfologi-Biomorphology Journal, 34(2): 134-142.

Majalah Biomorfologi (Biomorphology Journal) p.ISSN:0215-8833, e.ISSN: 2716-0920
doi: 10.20473/mbiom.v34i1.2024.134-142

Highlights

1. Biodentine has undergone thorough research as a bone grafting substance due to its ability to promote bone regeneration and effectively treat root or tooth fractures.
2. PRF and Biodentine can stimulate osteogenesis, affecting bone repair, particularly in mandibular fractures.

BACKGROUND

Mandibular fracture is one of the most common fractures. The most common cause of mandibular fractures is traffic accidents. An impact that is too hard on the face can result in a mandibular fracture. According to its location, the mandible is more prominent and easier to receive impact. Mandibular fracture is a condition where the mandibular bone is broken, which can be caused by facial trauma or pathological conditions. Fracture lines in the mandible often occur in areas of weakness. The thin area on the mandible is the angle and sub-condyle, which becomes a weak mandible area. In addition, weak points are also found in the mental foramen, the mandibular angle where the third molar teeth are located, and the mandibular condylar neck (Dewi, et al., 2022).

Numerous studies demonstrate that patients with mandibular fractures require an individualized approach in the selection of a treatment strategy due to the potential concomitance of different types of bone diseases, odontogenic infections, fractures in various regions of the mandible or simple skeleton, etc. (Diachkova, et al., 2022). The most common treatment for mandibular fractures is fixation. There are two mandibular fixation techniques, the closed and open techniques. In the closed technique, maxillomandibular fixation devices can achieve fracture immobilization and reduction. In an open procedure, the fractured part is opened surgically, and the fracture segment is reduced and fixed directly using a wire or plate called wire or plate osteosynthesis. These two techniques are only done separately but are sometimes applied in combination, which is called combined procedures (Dewi, et al., 2022).

Despite the high efficiency of modern treatment methods, bone tissue cannot be restored at total volume in all cases. Therefore, xenogenic agents are used to accelerate the reparative process. The basis of the stimulation of regeneration lies in induction, conduction, and regeneration due to the introduction of estrogenic cellular elements (stimulation by substitution). Each potency can stimulate reparative osteogenesis, either independently or in combination. The bone deficiency was filled with a collagen osteotropic substance (Diachkova, et al., 2022). PRF (Patelet-rich fibrin) is a human blood biomaterial consisting of platelet concentrate, fibrin matrix, cytokines, growth factors, and cells. Biodentine is a calcium-silicate-based material that has drawn attention in recent years and has been advocated to be used in various clinical applications, such as root perforations, apexification, resorptions, retrograde fillings, pulp capping procedures, and dentine replacement. Platelet-rich fibrin and dentine can stimulate bone osteoinduction when combined with bone grafts.

OBJECTIVE

This article aimed to compare the effect of osteoinductive platelet-rich fibrin with Biodentine for mandible fractures.

MATERIAL AND METHOD

This study used electronic databases such as PubMed, Scopus, ScienceDirect, Elsevier, and Google Scholar for research screening. It used specific keywords: mandibular fracture, mandibular fracture and xenograft, jawbone regeneration, mandibular bone regeneration, platelet-rich fibrin and jawbone regeneration, and Biodentine and jawbone regeneration. The paper was then filtered through a qualitative and quantitative selection.

Inclusion Criteria

The study was obtained based on literature studies in journals and textbooks published in the last ten years (2013-2023). The inclusion criteria deliberated human studies, in vitro and in vivo research, and reports. Off-topic publications were excluded from the analysis. Afterwards, the publications were categorized according to the surgical process and the study design.

Selection of the Studies

Independently qualified and expert reviewers screened the study data and analysis. After a primary check on the research title, every abstract of the identified papers was assessed as the 1st screening level. The full text of the included papers was obtained. Furthermore, they were classified for qualitative synthesis. The electronic database research has identified a total of 156 manuscripts. Forty-eight

duplicates have been deleted from the screening, and 108 papers have been considered for the full-text evaluation. Eleven full texts were not found, and 87 were out of topic to be excluded. Finally, ten papers have been incorporated into the analytical synthesis.

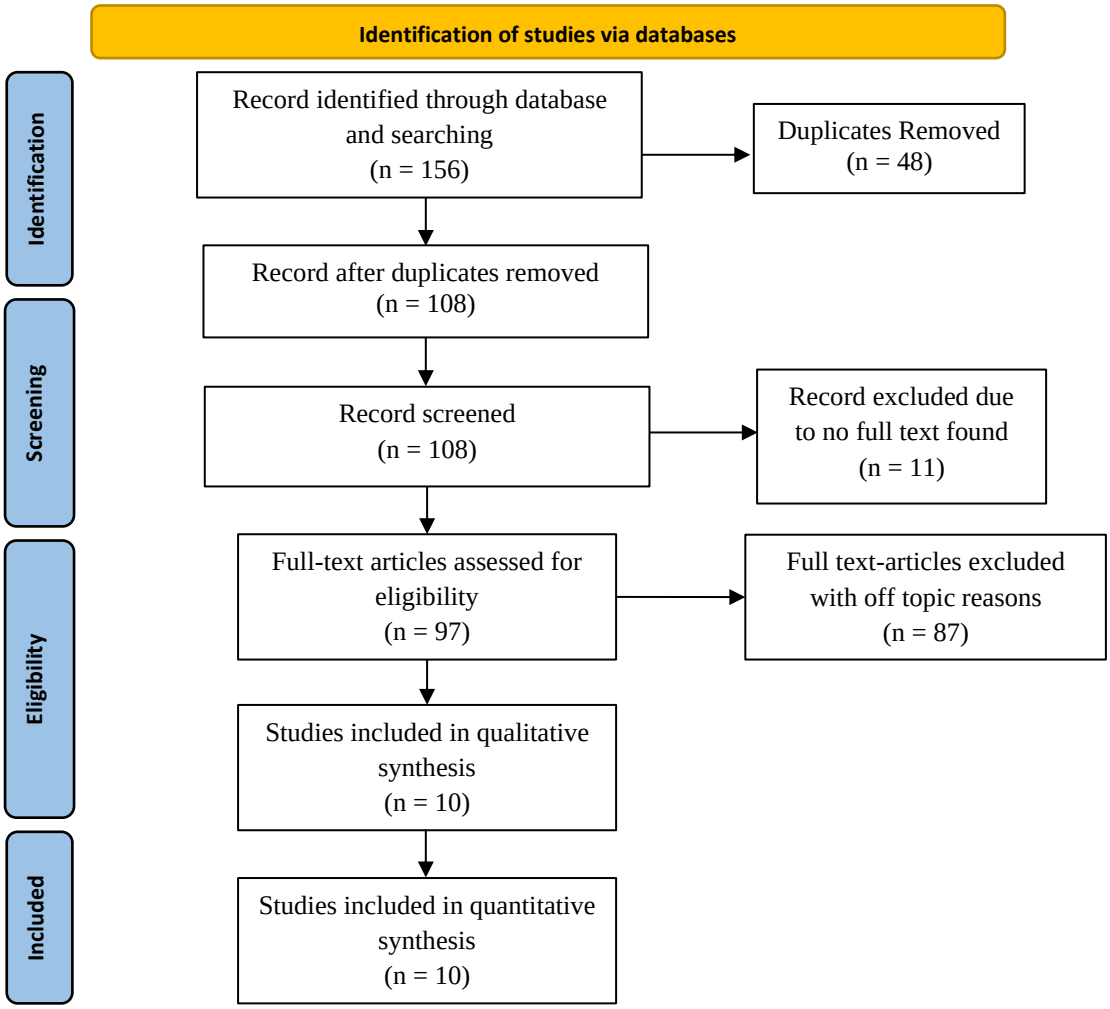

Figure 1. PRISMA flowchart of the study design and manuscript-selection process.

RESULT

Table 1 shows and summarizes the main effective results of the osteoinductive effect of platelet-rich fibrin and Biodentine.

Table 1. PICO Table of the osteoinductive mechanism of platelet-rich fibrin and Biodentine.

References	Population	Intervention	Comparison	Outcome
Hakam, et al., (2019)	Thirty adult male rabbits created bilateral bone defects in the right side of the mandibles as the experimental	The rabbits were divided into two groups. Group 1 was loaded with Biodentine on the experimental side.	Histological examination for bone defect specimens was prepared by light microscope and quantitative analysis of	Both Biodentine and nanobone had initiated osteogenesis. The Biodentine group showed spread-out braided bone trabeculae with entrapped osteocytes visible over one week. Several nuclei with different shapes and staining intensities were occasionally

	side and another side as the control.	Group 2 was packed with nanobones on the experimental side.	collagen one alpha and Runx-2 gene expression by real-time PCR.	detected in distinct lacunae. More bone spicules than in the preceding interval were visible after a two-month gap. Around the freshly produced trabeculae, it was clear that osteoblast-like cells were present to line their boundaries. However, Biodentine showed that the newly formed bone had apparently less quality than nanobone. It showed that nanobones had a more significant healing result.
Rajendran, et al., (2019)	Forty-five enamel specimens were extracted from human permanent premolars. It was free from defects, decay, and filling. All specimens were subjected to demineralization using McInnes demineralizing solution.	The specimens were divided into three groups. Group 1, as control, was treated with regular toothpaste. Group 2 was treated with toothpaste containing calcium sodium phosphosilicate. Group 3 was treated with a topical cream containing casein phosphopeptide—amorphous calcium phosphate.	The samples were then subjected to a scanning electron microscope to assess the topographical pictures and the surface changes seen on enamel; and energy dispersing X-ray analysis to estimate quantitatively the amounts of mineral in a given tooth sample after the remineralizing cycle.	Group I showed a mean calcium and phosphorus value of 65.32 ± 0.51 and 21.13 ± 0.75, respectively, for sound enamel. After remineralization with regular toothpaste, the mean calcium and phosphorus values obtained were 55.24 ± 0.49 and 15.76 ± 0.54, respectively, less than that of sound enamel and statistically significant ($p<0.001$). The values obtained after remineralization were similar to the mean calcium and phosphorus content of demineralized samples.
Gandolfi, et al., (2017)	Six mature male rabbits with four surgical bone defects on each side of the tibia.	The control group was left empty from 4 surgical defects, and another bone defect was filled with Biodentine, MTA Plus, and ProRoot MTA.	After 30 days, tibiae were retrieved and submitted to histological analysis and microchemical characterization using Optical Microscopy (OM) and Environmental Scanning Electron Microscopy with Energy Dispersive X-ray analysis (ESEM-EDX).	ESEM observed the histological section of a surgical defect treated with Biodentine. According to the histology findings, the surgical defect was filled by freshly produced bone trabeculae. The resected walls of the old cortical bone were in close contact with the new bone. Wide gaps could be seen, and little remnants of material were found. The bone that had just developed displayed significant mineralization. The osteoid matrix is being mineralized in some places.
Kaskos & Ali (2020)	Twenty rabbits with three surgical created monocortical defects on the mandible.	One surgical defect was left unfilled and considered as control, and the other defects were	CT scan was used as a parameter for bone density on follow-up dates 7, 14, 21, and 28	The impact of adding Biodentine on a rabbit's mandible defect was examined. By the seventh day, there was no significant disparity in bone density between dentine-treated (116.17 ± 1.41) and

		implanted with MTA and Biodentine.	days after operative surgery.	control (112.3 ± 1.36) samples. Similarly, by day 14, there was no significant variation in bone density between the Biodentine (153.43 ± 1.86) and control (148.2 ± 1.78) groups. However, by day 21, a notable distinction in bone density emerged, with Biodentine (359.27 ± 3.56) surpassing control (344.07 ± 4.08) insignificance. By the 28th day, Biodentine demonstrated a mean of 1012.07 ± 11.95.
Barczak, et al., (2020)	A literature review of 52 papers was selected, encompassing those directly focusing on Biodentine and other relevant papers not mentioning Biodentine but concerning dental materials in general.	No Intervention	No Comparison	Biodentine triggers the activation of cellular differentiation factors, such as osterix, which facilitates the differentiation of osteoblasts and thereby contributes to the generation of new bone. Biodentine also plays a role in regulating inflammatory responses and encourages the differentiation of both fibroblasts and osteoblasts. This process stimulates the creation of collagen bundles within the periodontal ligament and the matrix of bone.
Wang, et al., (2022)	Ten New Zealand white rabbits with separated SM-MSCs from the maxillary sinus.	The osteogenic differentiation ability of cells stimulated by PRF or osteoinductive medium was evaluated by alkaline phosphatase staining, alizarin red staining, PCR, and Western blot. As the bone graft materials for maxillary sinus floor elevation, an equivalent volume of Bio-oss and the mixture of Bio-oss and PRF were used.	To evaluate the the osteogenic effect, Micro-CT, bone double-staining, HE staining, Masson staining, and toluidine blue staining were used at 8 and 12 weeks post-surgery.	Due to the abundance of growth factors in PRF-conditioned medium, SM-MSC proliferation, migration, and osteogenic differentiation are all encouraged. Activating the ERK 1/2 signaling pathway, PRF-conditioned medium, OM, or the combination of the two above could enhance osteogenic differentiation. According to animal trials, using PRF significantly sped up the rate of new bone production in the maxillary sinus and increased the quantity and quality of newly created bone.
Augustina, et al., (2023)	Eighteen New Zealand rabbits with a critical bone defect surgery.	The rabbits were divided into three groups. Group 1 was treated by applying xenograft.	The results of the sample tissue were evaluated by immunohistochemical staining.	Lower MMP-13 expression was found in the PRF group. PRF possesses qualities that enhance graft position stability, bone regeneration, wound healing, and hemostasis. Controlled inflammation results in a drop in inflammatory mediators and a rise in osteoblast activity due to a

		Group 2 was treated by applying PRF. Group 3 was treated by combining the application of xenograft and PRF.		reduction in MMP-13 production. Growth factors and other mediators that promote bone regeneration and faster wound healing are concentrated in PRF.
Neiva, et al., (2016)	Eight beagle dogs with first molars extracted on both sides of the mandibular.	On one side of the mandibular tooth sockets, L-PRF was filled, and then the implant, covered by L-PRF in a membrane shape, was placed. Conversely, the implants were placed on the socket without applying L-PRF.	The tissue samples were prepared and then subjected to Stevenel's Blue and Van Gieson staining technique.	General histomorphologic observations for the implants placed along with L-PRF in fresh extraction sockets differed from those where no L-PRF was utilized primarily due to the lack of soft tissue migration through the gap formed between implant and socket wall, suggesting L-PRF's efficiency as a barrier during healing. The osteogenic potential and adequacy of L-PRF substituting the blood clot during socket healing were confirmed since bone growth occurred from the socket walls towards the implant, leading to substantial bone formation.
Ocak, et al., (2017)	Twenty-four adult sheep	Two adult sheep were used as group control. Group 1 was treated with a bovine and autogenous bone mixture. Group 2 was treated with PRF.	The specimens were evaluated by histologic and histomorphologic examination.	In the ninth month, the PRF group continued to exhibit observable recent bone development, accompanied by remaining traces of PRF. The newly generated bone within the PRF group exhibited greater density and increased firmness.
Pripatnanont, et al., (2015)	Twelve adult male New Zealand white rabbits on osteogenic periosteal distraction.	The rabbits were divided into four subgroups. A modified Hyrax device was performed in group 1. Group 2 was treated with a device and PRF. Group 3 was treated with PRF. Group 4 was treated with a sham.	The specimens were evaluated by performing analyses of radiographic, histological, histomorpho-metric, and micro-computed tomography (micro-CT).	During the 4-week and 8-week periods of bone consolidation, the PRF-treated group exhibited substantial healing of the defect, characterized by robust cortical and compact trabecular bone. Notably, the cortical bone in the 8-week group displayed greater thickness and density than the 4-week group. The highest percentages of bone volume and bone area were presented by group 2, which was treated with a device and PRF.

DISCUSSION

Following the nasal bone, the mandible is the facial bone that commonly experiences fractures. The separation of the mandibular bone is termed a mandibular fracture. Consequences of such fractures include airway destabilization, teeth misalignment, impaired joint function, discomfort, infection, and nerve sensation abnormalities (Karthik & Sanjay, 2015). In some complex cases, bone grafts with osteoinduction effects are used because the atrophic mandible's dense cortical bone and poor vascularity and marrow supply make it difficult for the area to recover (Shokri, et al., 2019).

Osteoinduction refers to bone grafts' capacity to prompt bone tissue creation by activating inactive osteoblasts from undifferentiated cells. Grafting materials possessing osteoconductive and osteoinductive properties provide a structure for existing osteoblasts and stimulate fresh osteoblast generation, potentially expediting graft integration (Suprianto, et al., 2019).

Biodentine, as a bone grafting material, has been extensively studied for its ability to enhance bone healing and its utility in addressing root or tooth fractures. Tang, et al., (2019) study investigated the impact of MTA and Biodentine on periarticular bone healing, revealing that Biodentine can actively promote bone healing. Daltoé, et al., (2016) observed that MTA and Biodentine could enhance indicators of mineralization. Furthermore, Ho, et al., (2018) utilized three-dimensional printed Biodentine/polycaprolactone composite scaffolds to demonstrate the potential of Biodentine in dental and bone regeneration. These investigations collectively affirm Biodentine's role in facilitating bone repair. Gandolfi, et al., (2017) research established that implanted Biodentine in bone defects led to the formation of new bone due to its ability to stimulate bone neoformation, differentiate osteoblasts, and promote angiogenesis.

The osteoinductive effect and mechanism of Biodentine in enhancing bone repair are likely correlated with the release of biologically active ions from calcium silicate cement (Gandolfi, et al., 2017). It was proposed that Biodentine would promote the proliferation and attachment of osteoblast-like cells, increasing their differentiation. These biological responses could be attributed to the material's topography and nano features, which provide a significant surface area for protein adsorption. Additionally, the production of bone matrix begins when osteogenic signals combine with soluble chemical cues, such as calcium, to cause a cascade of cellular development into osteoblast cell lines. According to a different theory, cements made of calcium silicate have an alkaline pH and can release calcium ions. These circumstances promote the activity of osteoblasts and the mineralization process by causing the nucleation of calcium phosphates and apatite (Hakam, et al., 2019). Calcium silicate cements release silicon, improving bone metabolism and speeding up new bone development. Also, calcium silicate cement is crucial in restoring bone by causing the calcium phosphate to precipitate at the periodontal tissue interface, causing a substantial rise in dentin gene expression for Runx-2. For osteoblastic development, matrix production, and mineralization during bone synthesis, Runx-2 is a key transcription factor (Rajasekharan, et al., 2018). This crucial gene is necessary for the production of osteoblasts and, as a result, the process of bone regeneration, as Runx-2 regulates osteoblast differentiation during the early stages of differentiation but suppresses it during the later stages (Kaskos & Ali, 2020).

On the contrary, platelet-rich fibrin (PRF) constitutes a composite comprising the cellular and molecular constituents essential for optimal healing. PRF represents the latest iteration of Platelet-rich plasma, a plasma from blood enriched with platelets housing a plethora of growth factors that encourage the mending of both bone and soft tissues (Augustina, et al., 2023). In some literature, it is stated that PRF can continuously increase the proliferation of all cell types, especially the proliferation of osteoblasts. This growth factor can regenerate bone tissue (Augustina, et al., 2023).

In in vivo or clinical studies, due to its abundant growth factors, PRF could be used as graft material. It can promote bone regeneration in extraction sockets, repair fractures, and improve gingival retreat. According to Neiva, et al., (2016), PRF can enhance bone formation within the socket arising from its compact fibrin network and cellular composition, which might enable a continuous discharge of growth factors. Furthermore, it can serve as a protective obstacle against soft-tissue growth. Growth factors generated by PRF significantly impact the volume and rate of new bone formation and the development of mature bone and thick bone trabeculae. In PRF, activated platelets may release various growth factors, including PDGF, VEGF, TGF-β, and IGF (Wang, et al., 2022). VEGF is the main factor that influences the differentiation of angiogenic cells and angiogenesis. It is the most powerful inducer of

angiogenesis, migration, and proliferation of endothelial cells. Controlling osteogenic growth factors through paracrine signals promotes osteogenesis and increases vascular permeability. VEGF can encourage bone regeneration in conditions such as radial segment defects in rabbits and femoral fractures in mice, resulting in good bone regeneration (Apte, et al., 2019). As the exclusive filling substance, the PRF membrane provides benefits such as simplified acquisition and application, the absence of supplementary grafting materials, immune responses, and stimulation of bone generation (Ocak, et al., 2017).

These two bone graft materials have their respective advantages and mechanisms when compared. PRF and Biodentine can induce osteogenesis, which impacts bone healing, especially mandibular fractures.

Strength and limitations

This study can be used as a reference point for further research endeavours. Studies have demonstrated that PRF and Biodentine can effectively promote bone regeneration. Currently, no research investigates the distinct osteoinduction mechanisms of PRF and Biodentine in managing mandibular fractures.

CONCLUSION

Osteoinduction is a necessary process that can enhance bone repair. Bone grafts with additional materials such as PRF or Biodentine may have a role in supporting soft tissue healing or bone regeneration. Biodentine facilitates bone repair by promoting the proliferation and attachment of osteoblast-like cells and increasing their differentiation. PRF is enriched with platelets containing a plethora of growth factors that encourage the mending of both bone and soft tissues. In this literature, there was no significant difference in the osteoinduction effect of PRF and Biodentine, even though these materials have different mechanisms of action for bone repair.

Acknowledgment

The authors thank the Department of Oral & Maxillofacial Surgery, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.

Conflict of Interest

All authors have no conflict of interest.

Funding Disclosure

None.

Author Contribution

MAN contributes to the conception and design, analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, provision of study materials or patients, and collection and assembly of data. IM and AJK contribute to the critical revision of the article for important intellectual content and final approval.

REFERENCES

Apte, R. S., Chen, D. S., Ferrara, N. 2019. VEGF in signaling and disease: Beyond discovery and development. Cell, 176(6): 1248–1264. doi: 10.1016/j.cell.2019.01.021.

Augustina, E. F., Rivai, N. R., Gifary, H. M. 2023. The expression of matrix metalloproteinase-13 (MMP-13) on xenograft and PRF in bone regeneration. Brazilian Dental Science, 26(2): 1–7. doi: 10.4322/bds.2023.e3366.

Barczak, K., Palczewska-Komsa, M., Sikora, M., et al. 2020. Biodentine™ – use in dentistry. Literature review. Pomeranian Journal of Life Sciences, 66(2): 39–45. doi: 10.21164/pomjlifesci.666.

Daltoé, M. O., Paula-Silva, F. W. G., Faccioli, L. H., et al. 2016. Expression of mineralization markers during pulp response to Biodentine and mineral Trioxide Aggregate. Journal of Endodontics, 42(4): 596–603. doi: 10.1016/j.joen.2015.12.018.

Dewi, N. P., Susanti, M., Vani, A. T., et al. 2022. Dextra mandibular fractures in traffic accident

patients. Jurnal Kewarganegaraan, 6(4): 7093–7099. Available at: http://repo.unbrah.ac.id/id/eprint/353/1/057.+Fraktur+Mandibula+Dextra+pada+Pasien+Kecelakaan+Lalu+Lintas (1).pdf.

Diachkova, E. Y., Popova, S. V., Arazashvili, L. D., et al. 2022. Complicated mandible fracture treatment with xenogenic bone graft. Applied Sciences, 12(5): 2384. doi: 10.3390/app12052384.

Gandolfi, M. G., Iezzi, G., Piattelli, A., et al. 2017. Osteoinductive potential and bone-bonding ability of ProRoot MTA, MTA Plus and Biodentine in rabbit intramedullary model: Microchemical characterization and histological analysis. Dental Materials, 33(5): 221–238. doi: 10.1016/j.dental.2017.01.017.

Hakam, H., Abdel Moneim, R., El Deeb, M. 2019. Osteoinductive potential and bone healing capacity of nanocrystalline Hydroxyapatite (nHA) versus Biodentine of surgically created defects in rabbits. Alveolar process (an animal study). Egyptian Journal of Histology, 902-917. doi: 10.21608/ejh.2019.16561.1164.

Ho, C. C., Fang, H. Y., Wang, B., et al. 2018. The effects of Biodentine/polycaprolactone three-dimensional-scaffold with odontogenesis properties on human dental pulp cells. International Endodontic Journal, 51(S4). doi: 10.1111/iej.12799.

Karthik, R., Sanjay, P. 2015. Incidence, aetiology and pattern of mandibular fractures in Pondicherry. Journal of Evolution of Medical and Dental Sciences, 4(104): 16946–16950. doi: 10.14260/jemds/2015/2555.

Kaskos, H. H., Ali, A. 2020. Facilitating osteogensis of Mineral Trioxide Aggregate and Biodentine by autogenous bone marrow (experimental study on rabbit). Journal of Duhok University, 23(1): 144–148. doi: 10.26682/sjuod.2020.23.1.15.

Neiva, R. F., Gil, L. F., Tovar, N., et al. 2016. The synergistic effect of leukocyte platelet-rich fibrin and micrometer/nanometer surface texturing on bone healing around immediately placed implants: An experimental study in dogs. BioMed Research International, 2016: 1–9. doi: 10.1155/2016/9507342.

Ocak, H., Kutuk, N., Demetoglu, U., et al. 2017. Comparison of bovine bone-autogenic bone mixture versus platelet-rich fibrin for maxillary sinus grafting: Histologic and histomorphologic study. Journal of Oral Implantology, 43(3): 194–201. doi: 10.1563/aaid-joi-D-16-00104.

Pripatnanont, P., Balabid, F., Pongpanich, S., et al. 2015. Effect of osteogenic periosteal distraction by a modified Hyrax device with and without platelet-rich fibrin on bone formation in a rabbit model: A pilot study. International Journal of Oral and Maxillofacial Surgery, 44(5): 656–663. doi: 10.1016/j.ijom.2014.12.004.

Rajasekharan, S., Martens, L. C., Cauwels, R. G. E. C., et al. 2018. Biodentine™ material characteristics and clinical applications: A 3 year literature review and update. European Archives of Paediatric Dentistry, 19(1): 1–22. doi: 10.1007/s40368-018-0328-x.

Rajendran, R., Kunjusankaran, R. N., Sandhya, R. N., et al. 2019. Comparative evaluation of remineralizing potential of a paste containing bioactive glass and a topical cream containing Casein Phosphopeptide-Amorphous Calcium Phosphate: An in vitro study. Pesquisa Brasileira em Odontopediatria e Clínica Integrada, 19(1): 1–10. doi: 10.4034/PBOCI.2019.191.61.

Shokri, T., Misch, E., Ducic, Y., et al. 2019. Management of complex mandible fractures. Facial Plastic Surgery, 35(6): 602–606. doi: 10.1055/s-0039-1700878.

Suprianto, K., Hidayati, H., Nilam, C., et al. 2019. Eggshell hydroxyapatite as a potential bone graft in periodontal therapy. MKGK (Majalah Kedokteran Gigi Klinik), 5(3): 76–87. doi: 10.22146/mkgk.65729.

Tang, J., Shen, Z. S., Qin, W., et al. 2019. A comparison of the sealing abilities between Biodentine and MTA as root-end filling materials and their effects on bone healing in dogs after periradicular surgery. Journal of Applied Oral Science, 27. doi: 10.1590/1678-7757-2018-0693.

Wang, J., Sun, Y., Liu, Y., et al. 2022. Effects of platelet-rich fibrin on osteogenic differentiation of Schneiderian membrane derived mesenchymal stem cells and bone formation in maxillary sinus. Cell Communication and Signaling, 20(1): 88. doi: 10.1186/s12964-022-00844-0.

FACULTY OF MEDICINE - UNIVERSITAS AIRLANGGA
MBIOMJ
MAJALAHBIOMORFOLOGI
(BIOMORPHOLOGY JOURNAL)
p-issn. 0215-8833 e-issn. 2716-0920
HTTPS://E-JOURNAL.UNAIR.AC.ID/MBIO

MBIOMJ

Indexed in:

Address:
The Unit of Journal Consortium and Folia Medica Indonesiana
Faculty of Medicine, Universitas Airlangga
Jl. Mayjen. Prof. Dr. Moestopo 47, Surabaya 60132, Indonesia
https://e-journal.unair.ac.id/MBIO

9 772716 092006