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Knowledge on and Compliance with Standard Precautions among Medical Students in Jharkhand, India: A Cross-Sectional Study

Ankita Jawanpuria, Chinmaya Dash

Department of Microbiology, Manipal Tata Medical College, Manipal Academy of Higher Education, Manipal, Karnataka, India

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Correspondence:
Dr. Ankita Jawanpuria,
Department of Microbiology,
Manipal Tata Medical College,
Manipal Academy of Higher Education, Manipal, Karnataka, India
Email: ankita.jpuria@gmail.com

Abstract

Objective: To assess the knowledge, attitude, and compliance with standard precautions among medical students in a tertiary care hospital in Jharkhand, India.

Methods: A total of 280 medical students, including phase I and phase II MBBS students, participated in the study. Phase I comprised an introduction to standard precautions in four-hour classes while the Phase II comprised of 8 hours of theory and three skill assessments on standard precautions according to the CBME curriculum and pandemic module. A structured validated questionnaire was used to collect data on the student's knowledge, attitude, and practice related to standard precautions. The questionnaire included items assessing various aspects of standard precautions, and scoring criteria were used to evaluate student performance. The overall level of knowledge, attitude, and practice was classified as poor (<50% score), moderate (50–79% score), and good (≥80% score).

Results: Among the phase I students, 32.8% got a good score. In contrast, in the phase II, 56.6% demonstrated good performance in adhering to standard precautions. The median total score was 68 (interquartile range 62–71) of 85, indicating a satisfactory level of understanding and compliance with standard precautions. However, areas such as needing to remove PPE before leaving the patient's environment and not recapping needles showed rooms for improvement.

Conclusion: Students showed good knowledge and compliance with standard precautions. Phase II students demonstrated a better understanding of standard precautions compared to their phase I counterparts. This highlights the positive impact of practical hands-on training as particularly emphasized in the phase II curriculum.

Keywords: Attitude, infection control, knowledge, medical education, medical students, standard precautions

Introduction

Healthcare-acquired infections (HAIs) are not typically present or in incubation at the time of admission. These infections are usually acquired 48 hours after the admission to the hospital.[1]

These infections pose significant morbidity and mortality among hospitalized patients worldwide. On average at any given time, 7% of total hospitalized patients in developed countries and 10% in developing countries acquire at least one HAIs. Death occurs in about 10% of affected patients.[2]

In 1996, the US Centers for Disease Control and Prevention (US CDC) introduced the Standard Precautions guidelines that summarize strategies to prevent infection transmission in healthcare settings. The standard precautions assume blood and body substances of all patients as potential infection sources, regardless the diagnosis or

presumed infectious status. The components of standard precautions include hand hygiene, injection safety, use of personal protective equipment (PPE), environmental cleanliness, waste management, respiratory hygiene, and cough etiquette.[3] All these precautions are also included in the minimum requirements for infection prevention and control programs. However, despite the fact that the Standard Precautions are implemented widely by health organizations, gaps in their implementation have been noted. Studies have revealed that medical students in teaching hospitals also spread infections because they are not aware of these precautions.[4-7]

Currently, early clinical exposure (ECE) and pandemic modules in the Competency-Based Medical Education (CBME) curriculum are being introduced in order to enhance the students' knowledge and practice of standard precautions. In this study, the knowledge and compliance of standard precautions among medical students in a tertiary care hospital, as well as the importance of teaching pandemic modules to students, was studied.

Methods

This was a cross-sectional study done within a period of three months (May to July 2023) in Manipal Tata Medical College. Phase I and Phase II MBBS students of the tertiary care hospital were assessed for their knowledge, attitude, and practice (KAP) of standard precautions, including hand hygiene, injection safety, use of personal protective equipment, respiratory hygiene, and cough etiquette, using a researcher-made, self-administered questionnaire that was developed based on the US CDC and WHO guidelines.[8-10]

The questionnaire was disseminated online through a professional network using Microsoft Teams forms with a single registration for each student. The questionnaire included a knowledge domain with 17 items with yes/no answer options; an attitude domain with 11 items, using a likert scale with five options: "strongly disagree (1)," "disagree (2)," "neutral (3)," "agree (4)," and "strongly agree (5)"; and the practice domain included 13 items with yes/no/not applicable options. The questionnaire was pre-validated by four experts with more than 5 years of experience in medical education. Construct and chances of misinterpretation were taken into account during validation. A disclaimer was given with the questionnaire that the marks of this study would not be disclosed. Filling out the form would be taken as consent. The participation in the study was voluntary, and a pilot testing was done on ten students to check the comprehensiveness of the questionnaire.

The non-probability sampling technique was applied and all MBBS Phase I and Phase II students were included in this study (n=298). Students who did not submit answers to the questionnaire were excluded. Eventually, 137 students of Phase I and 143 students of Phase II MBBS filled out the complete form, making a sample size of 280. Phase I was introduced to standard precautions in four-hour classes, while Phase II was given approximately eight (8) hours of theory and three skill assessments on standard precautions according to the CBME curriculum and pandemic module.

Ethical clearance was obtained from the institutional ethical committee under the ethical clearance no. MTMC/IEC/2023/03. Data were analyzed using Jamovi 2.3.24, and descriptive analysis results were presented in tables reporting percentages for categorical data and median with interquartile range for continuous data. The overall level of knowledge, attitude, and practice was classified as poor (<50% score), moderate (50–79% correct answers), and good (80–100% score).

Results

A total of 280 medical students participated in this study, which accounted for 94% of the total population of eligible students. Among these respondents, 60% were female and 40% were male, with a median age of 21 years (ranging from 18 to 25 years). In terms of knowledge of standard precautions, 44.6% of the students correctly answered ≥80% of the knowledge statements. Examples of questions and the percentage of students who gave correct answers are shown in Fig. 1 and 2.

In terms of attitude, the majority of students strongly agreed that "hand hygiene should be done before and after patient interventions" (85.3%), "standard precautions are effective in preventing infection spread" (82.5%), and "healthcare providers should keep adequate protective barriers" (86.0%). Nevertheless, only 61.1 % of students strongly agreed that "standard precautions are easy to follow" and few students thought that "PPE should not be used while there are emergencies" (15.7%). Some students strongly agreed that "PPE may harm patients psychologically" (10.3%) and "it is not logical to assume all patients are contagious unless infection is confirmed"

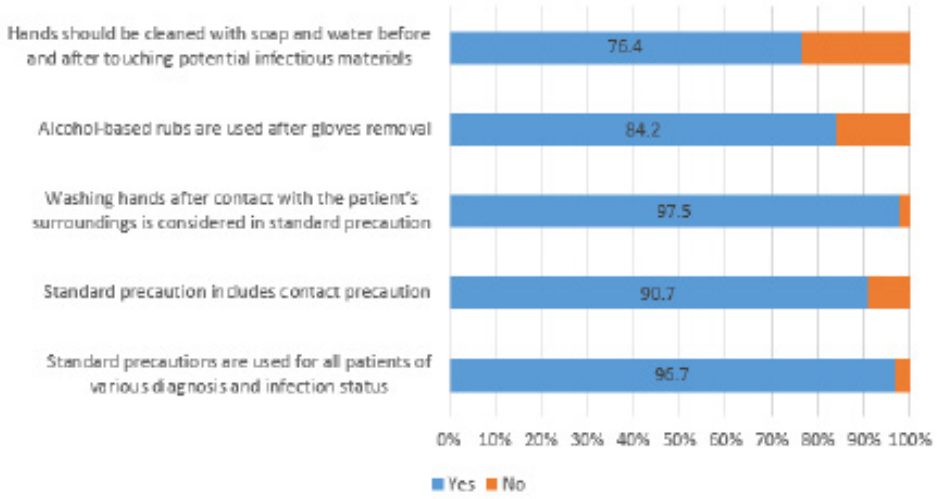

Fig. 1 Example of Questions Related to Hand Hygiene and Percentages of Students' Answers

(27.1%). In practice, approximately half of the students (47%) demonstrated good adherence to standard precautions. Examples of findings in this domain are given in Table 1.

Figure 2 presented the students' practice in implementing standard precautions. The X-axis shows the performance of students and the Y-axis denotes the percentage of students.

Overall, these students showed moderate performance, with 44.6%, 43.9%, and 47% of students achieving a score ≥80% in the knowledge, attitude, and practice sections, respectively. The median total score for students was 68 (interquartile range 62–71) out of 85, indicating good understanding and compliance with standard precautions. A comparison of Phase I and Phase II students is shown in Figure 3.

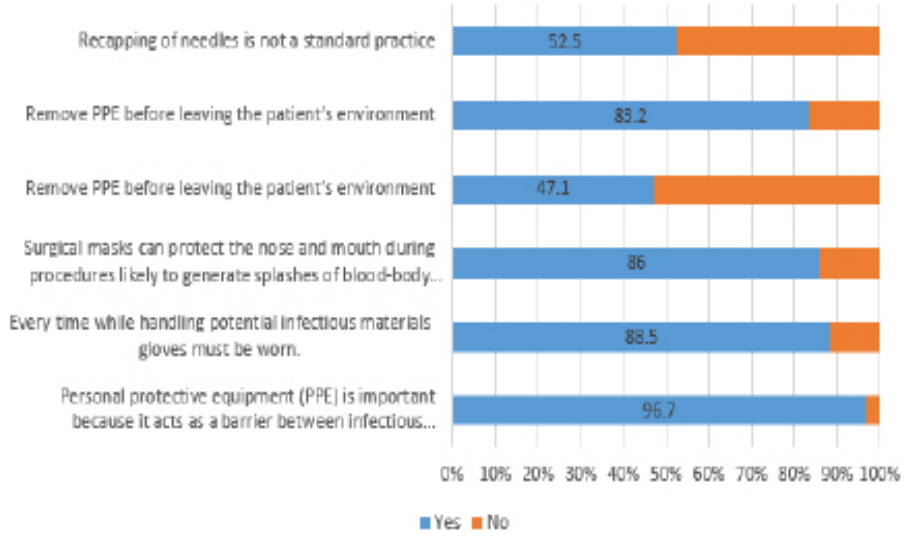

Fig. 2 Example of Questions Related to PPE and Needle Stick Injury Practices with Percentage of Students' Answers

Table 1 Practice Questions and Their Adherence by Student

Practice Questions	Adherence
I always perform hand hygiene when coming in contact with patients	78.2%
I always wear gloves when touching the patient's mucosa	86.4%
I always wear a mask when I observe any procedures that might lead to the spraying of blood, body fluids, secretions, or excretions	87.1%
Hands should be washed after coughing or sneezing	49.6%
I always wear a single-use surgical mask and discard it after each use	24%

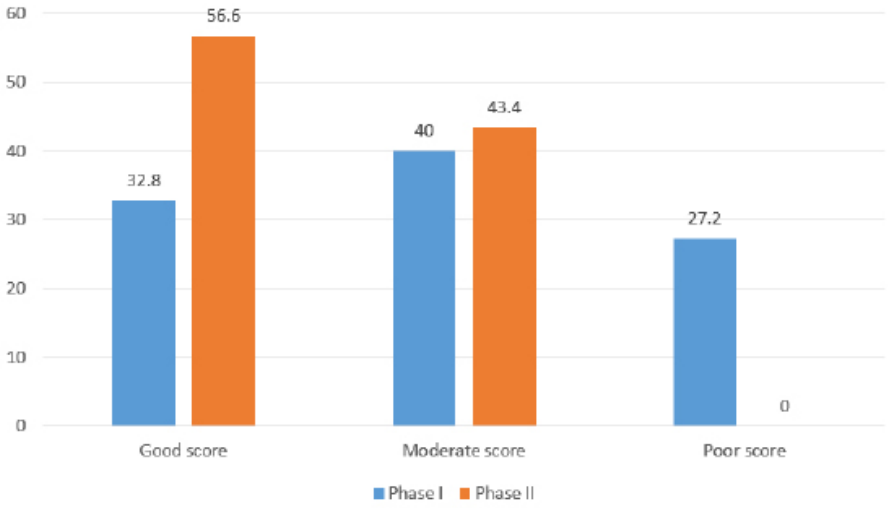

Fig. 3 Comparison of Phase I and Phase II Students

Discussion

This study was conducted among phase I and phase II medical undergraduate students of a private medical college in India. The findings of the study revealed both positive aspects and areas requiring improvement in these future healthcare providers.

In terms of knowledge, the study showed that 44.6% of the students demonstrated good knowledge of standard precautions. This indicates a moderate level of understanding in a significant proportion of the participants. The high scores in areas such as hand hygiene, personal protective equipment (PPE) use, disposal of sharps, and reporting needle stick injuries suggest that these topics are adequately covered in the curriculum or clinical training. However, areas such as the need to remove PPE before leaving the patient's environment and the practice of not recapping needles showed rooms for improvement.

In a study by Ibrahim *et al*., the knowledge of medical students regarding standard isolation precautions is also demonstrated to be very low (48.44%), which is similar to this study.[4] Saati *et al*. showed a good levels of knowledge representing 71.0% of medical undergraduates understanding on infection control measures.[5] In terms of attitude, the majority of students (85.3%) exhibited a positive attitude towards performing hand hygiene, recognizing the effectiveness of standard precautions in preventing infection spread and ensuring adequate protective barriers. This is an encouraging finding as a positive attitude is essential for the consistent implementation of infection prevention practices. However, a considerable number of students have expressed concerns about the difficulty of following standard precautions, questioning the necessity of using PPE in emergencies (15.7%) and suggesting potential psychological harm to patients due to PPE use (10.3%).

In terms of practice, the study showed that

47% of the students exhibited good adherence to standard precautions. This indicates a moderate level of compliance with infection prevention practices among the participants. Consistent hand hygiene, proper glove use, and appropriate mask usage are reported by a significant proportion of students. However, there are rooms for improvement in certain areas, such as washing hands after coughing or sneezing. Emphasizing the importance of proper hand hygiene practices in all situations, including after respiratory events, can help address this gap. In a study by Saati *et al*., 55.7% of medical undergraduates showed good practice levels of standard precautions.[5]

The present study revealed that 100% of phase II and 72.8% of phase I medical students demonstrated good to moderate knowledge of standard precautions. Phase II students demonstrated a better understanding of standard precautions compared to their phase I counterparts. This highlights the positive impact of practical hands-on training, particularly emphasized in the phase II curriculum. A multicentric study would have provided better comparisons. In addition, the self-reported design in this study may introduce bias. Thus, future research could involve direct observation to measure compliance more accurately.

In conclusion, the findings of this study suggest that the inclusion of early clinical exposure and pandemic modules in the Competency-Based Medical Education (CBME) curriculum for medical students has positively influenced students learning on standard precautions in healthcare.

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Original Article

Factors affecting Umbilical Cord Vitamin D Concentration and Its Association with Maternal Vitamin D Level

Aditya Aggarwal, Amit Kumar Modi, K. Rimi Singh, Avinash Kumar Jha

Department of Paediatrics, Noida International Institute of Medical Sciences (NIIMS), Noida International University, Uttar Pradesh, India

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Correspondence:
Dr. Aditya Aggarwal
Assistant Professor,
Department of Paediatrics,
Noida International Institute of Medical Sciences (NIIMS), Noida International University, Uttar Pradesh, India
E-mail: Aditya.aggarwal.26@gmail.com

Abstract

Objective: To analyze factors affecting maternal and umbilical cord levels of vitamin D and to understand the correlation between maternal and umbilical cord vitamin D levels.

Methods: This was a cross-sectional study conducted at the department of pediatrics of Dr. D. Y. Patil Medical College and Research Centre, Pune, India. Maternal and umbilical cord vitamin D levels were examined in 300 pairs of mother and child over a period of two years. Informed consent was obtained from all participants. The vitamin D level was measured using chemiluminescent immunoassay and classified as either deficient, insufficient or adequate depending on specific cut-offs. Correlations between maternal and neonatal vitamin D levels and demographic factors like religion, socioeconomic status, and sun exposure were also exlored. Statistical tests were performed using the SPSS 21.0 software, with $p<0.05$ deemed p-valueas significant.

Results: There was significantly high prevalence of vitamin D deficiency in neonates, (78.67%). Key factors influencing maternal vitamin D levels were religion ($p=0.027$), maternal education ($p=0.003$), gravida status ($p=0.035$), and sunlight exposure, with sunlight exposure showing a very strong correlation to the deficiency ($p<0.001$). Moreover, maternal serum calcium levels significantly affected vitamin D status ($p<0.001$). A significant association was observed between maternal and cord blood vitamin D levels, with the maternal vitamin D level strongly predicted vitamin D status in neonates ($p<0.001$).

Conclusion: The umbilical cord vitamin D level strongly correlates with the maternal vitamin D level, which is significantly affected by maternal education, residence, pregnancy status, gestational age, and sun exposure.

Keywords: Fetal development, neonate, pregnancy, vitamin D

Introduction

Vitamin D is a fat-soluble vitamin (secosteroid) obtained by the human body in two ways: through dietary intake, mainly from fatty fish, eggs, and fortified foods, and through endogenous production in the skin after ultraviolet-B exposure. This vitamin involved in the uptake and degradation of calcium and phosphorus in bones from serum. In addition, vitamin D has been shown to play a role in cell differentiation, cell growth, metabolism, and immunity. There is an increasing interest in analyzing the role of vitamin D in these non-classical functions.[1]

A population of particular interest in the emerging vitamin D narrative is the pregnant women. The role of vitamin D during pregnancy is particularly crucial due to the dependence of the growing fetus on its mother for sufficient vitamin D.[2] Since vitamin D levels tend to be lower during pregnancy than in comparable non-pregnant women.[3], there is controversy surrounding the optimal vitamin D levels for

the fetus, its effects on various outcomes, and the relationship between maternal and fetal vitamin D. During pregnancy, maternal serum concentrations of 25(OH)D3 correlate with dietary vitamin D intake as well as vitamin D supplementation.[4]

It has been demonstrated that the maternal serum concentration of 1,25-dihydroxyvitamin D_3, the circulating and active form of vitamin D is significantly elevated during pregnancy.[5] During fetal intrauterine development, the production of the 1,25-dihydroxyvitamin D3 is primarily done by the placental decidual cells, which facilitates enhanced calcium absorption. The fetus relies entirely on maternal 25(OH) D3, which is thought to cross the placenta. After birth, both full-term and preterm neonates experience hypocalcemia, which, along with increased secretion of parathyroid hormone, stimulates the production of 1,25-dihydroxyvitamin D.

Adequate intake of vitamin D is important during pregnancy due to possible associations between maternal vitamin D levels and fetal and infant outcomes. Insufficient or deficient levels of vitamin D in mothers have been linked to an increased risk of gestational diabetes and preeclampsia.[7] Also, vitamin D deficiency during pregnancy has been associated with various negative health outcomes in offsprings, including a higher incidence of abortion, low birth weight, neonatal hypocalcemia, impaired development, and rickets.[8]

Many studies have reported a significant association between cord blood vitamin D levels and maternal serum vitamin D levels; however, it remains unclear how strong these two are interrelated. Furthermore, there is a lack of studies regarding these associations, particularly in the Indian population. If a significant association between maternal vitamin D levels and cord vitamin D levels can be established, it could have important implications for both maternal and child health. These findings could be crucial for a country like India, where infant mortality rates are high, in its progress toward achieving Millennium Development Goals in these parameters.[9]

This study was undertaken to analyze the factors affecting both maternal and cord vitamin D levels and to determine the correlation between them

Methods

It was a cross-sectional study carried out in the Department of Pediatrics at Dr. D. Y. Patil Medical College and Research Centre, Pimpri, Pune, India. The duration of the study was 2 years, extending from December 2021 to November 2023. The study comprised healthy women aged between 18 and 40 years and their newborns delivered at this institute, with all mothers providing informed and written consent to participate.

Exclusion criteria included refusal to give consent, mothers below 18 years or above 40 years, and those with a history of thyroid or parathyroid hormone abnormalities, chronic renal failure, or chronic liver disease. Informed and written consent was obtained from all participants. The sample size was calculated based on pilot studies conducted on the estimation of cord vitamin D in newborns. Considering a 90% power and a 95% confidence interval, the required sample size was determined to be 250 samples. Based on the central limit theorem, a sample size of more than 250 was assumed to be sufficient. Thus, 300 blood samples were included in this study. A detailed maternal history was obtained to identify the presence of disorders known to affect maternal serum calcium levels, such as thyroid and parathyroid diseases, chronic renal failure, or liver diseases. After ruling out any significant maternal illness, 300 pairs of cord blood samples and maternal blood samples were collected from mothers who met the inclusion criteria and provided written consent after the procedure was fully explained to them. For each neonate, 3 mL of blood from the umbilical cord was collected in an EDTA-containing tube, then centrifuged at 3,000 rpm for 10 minutes. The serum was separated and stored as 1 mL aliquots at −20°C until analysis; the same process was followed for the maternal samples. The serum levels of 25-hydroxy vitamin D were measured using chemiluminescent immunoassay (CLIA). Vitamin D levels were categorized based on the following cut-offs: below 30 nmol/L as deficient, 30–50 nmol/L as insufficient, and above 50 nmol/L as adequate.

The correlation between cord blood and maternal vitamin D levels was examined. Additionally, the variation of vitamin D levels with different gestational ages and neonatal birth weights was assessed. The relationship between maternal and neonatal vitamin D levels and various demographic and lifestyle factors—including the mother's religion, habitat, socioeconomic status, education, number of pregnancies, parity, sun exposure, and dietary habits—was also analyzed.

Data were presented as mean ± standard

Table 1 Mean Age of Mothers and Its Correlation with Maternal Levels of Vitamin D

		Deficient	Insufficient	Sufficient	p-value
Age	Mean ± SD	24.58±3.03	25.11±3.65	25.25±4.37	0.483
	Min.-max.	19–35	20–35	21–32	

deviation (X±SD) or percentage (%). Linear correlation and regression were used to test the relationships between the measured parameters. Cut-off values were calculated from the receiver operating characteristics (ROC) curve as mean±2 SD of the control group. For comparisons of quantitative data between groups, an unpaired t-test was applied. Data were tabulated and statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 21.0 software. A p-value of less than 0.05 was considered statistically significant.

Results

The maternal ages of women whose babies were sampled for cord vitamin D levels ranged from 19 to 35 years. The mean age for the deficient group was 24.58±3.03 years, for the insufficient group it was 25.11±3.65 years, and for the sufficient group it was 25.25 ± 4.37 years. The mother's age as a factor influencing variability in cord blood vitamin D levels was found to be statistically insignificant (p= 0.483) (Table 1).

The analysis of cord vitamin D levels in neonates showed that the majority, 236 (78.67%), were found to be deficient in cord blood vitamin D at the cut-off point of serum calcidiol levels (25(OH)D) <20 ng/mL (< 50 nmol/L). Out of a total of 300 cord blood samples, only 8 had sufficient vitamin D levels >30 ng/mL (>75 nmol/L), while 56 samples had insufficient levels between 20–30 ng/mL (50–75 nmol/L) (Table 2).

Analysis on the factors affecting maternal vitamin D levels revealed a significant correlation with religion (p-value of 0.027). Hindu participants showed higher sufficiency rates (6.8%) compared to Muslims, who had no sufficient cases. However, the area of residence did not show a significant correlation with vitamin D levels, as indicated by a p-value of 0.938, suggesting that living in either rural or urban areas does not significantly affect vitamin D status. Maternal education approached significance (p-value of 0.051), indicating a potential influence, with higher education groups showing lower sufficiency rates. Socioeconomic status, with a p-value of 0.090, also suggested a possible effect but was not statistically significant, indicating minor variations across economic groups. Maternal gravida status (p-value of 0.027) showed a notable distinction, with primigravida mothers having a higher sufficiency rate (10.8%) compared to multigravida mothers (3.2%), highlighting how the number of pregnancies may affect vitamin D levels. Conversely maternal parity (p-value of 0.584) showed no significant influence, indicating that parity does not significantly alter vitamin D levels. Dietary habits of the mother were not significantly correlated with vitamin D levels (p-value of 0.093), although vegetarian mothers exhibited slightly higher sufficiency (9.6%). A strong correlation was observed with maternal exposure to sunlight, evidenced by a highly significant p-value of <0.001; inadequate sun exposure resulted in a 94.9% deficiency rate. Gestational age and birth weight both showed no significant differences in vitamin D levels, with p-values of 0.093 and 0.908, respectively (Table 3).

Table 2 Status of Cord Vitamin D Levels

Cord Vitamin D Level	Frequency (n=300)	%
Deficient	236	78.67
Insufficient	56	18.67
Sufficient	8	2.67

The study of various factors influencing cord blood vitamin D levels among the cases examined revealed significant findings for several variables. Religion (p-value=0.151) and socioeconomic status (p-value=0.597) did not significantly impact vitamin D levels, despite differences in deficiency rates between religious groups and across socioeconomic categories. In contrast, area of residence (p-value=0.026) demonstrated a notable difference, with rural residents experiencing higher deficiency rates than urban dwellers. Maternal education significantly influenced vitamin D status (p-value=0.003), with higher education levels correlating with better vitamin D levels. Maternal gravida status (p-value=0.035) and gestational age (p-value

=0.046) also demonstrated significant associations, indicating that physiological changes during pregnancy affect vitamin D synthesis. Meanwhile, maternal parity (p-value=0.819) and the gender of the neonate (p-value=0.390) did not present significant differences. Dietary habits did not significantly affect the vitamin D levels (p-value=0.080). Most strikingly, inadequate maternal sun exposure (p-value <0.001) resulted in a 100%

Table 3 Factors Affecting Maternal Serum Vitamin D Levels

Factors Affecting Maternal Serum Vitamin D Levels		Total (n=300)	Maternal Serum Vitamin D Levels			p-value
			Deficient (n=236) (%)	Insufficient (n=56) (%)	Sufficient (n=18) (%)	
Religion	Hindu	266	167 (62.8)	81 (30.5)	18 (6.8)	0.027
	Muslim	34	29 (85.3)	5 (14.7)	0 (0.0)	
Area of residence	Rural	157	104 (66.2)	44 (28)	9 (5.7)	0.938
	Urban	143	92 (64.3)	42 (29.4)	9 (6.3)	
Maternal education	Primary	19	15 (78.9)	4 (21.1)	0 (0.0)	0.051
	Secondary	61	35 (57.4)	19 (31.1)	7 (11.5)	
	Senior secondary	85	55 (64.7)	26 (30.6)	4 (4.7)	
	Graduate	101	61 (60.4)	33 (30.6)	7 (4.7)	
	Above	34	30 (88.2)	4 (11.8)	0 (0.0)	
Socio economic status of the family	Lower	2	2 (100.0)	0 (0.0)	0 (0.0)	0.090
	Upper lower	82	57 (69.5)	20 (24.4)	5 (6.1)	
	Lower middle	86	46 (53.5)	36 (41.9)	4 (4.7)	
	Upper middle	124	85 (68.5)	30 (24.2)	9 (7.3)	
	Upper	6	6 (100)	0 (0.0)	0 (0.0)	
Maternal gravida	Primigravida	111	59 (53.2)	40 (36)	12 (10.8)	0.027
	Multigravida	189	137 (72.5)	46 (24.3)	6 (3.2)	
Maternal parity	Nulliparous	238	154 (64.7)	68 (28.6)	16 (6.7)	0.584
	Multiparous	62	42 (67.7)	18 (29)	2 (3.2)	
Maternal dietary habits	Vegetarian	114	74 (64.9)	29 (25.4)	11 (9.6)	0.093
	Non-vegetarian	186	122 (65.6)	57 (30.6)	7 (3.8)	
Maternal exposure to sun	Inadequate	117	111 (94.9)	6 (5.1)	0 (0.0)	<0.001
	Adequate	183	85 (46.4)	80 (43.7)	18 (9.8)	
Gestational age	Pre-term	79	52 (65.8)	21 (26.6)	6 (7.6)	0.093
	Term	152	104 (68.4)	41 (27)	7 (4.6)	
	Post-term	38	24 (63.2)	14 (36.8)	0 (0.0)	
	Post	31	16 (51.6)	10 (32.3)	5 (16.1)	
Birth weight	Very low	29	19 (65.5)	8 (27.6)	2 (6.9)	0.908
	Low	69	47 (68.1)	20 (29)	2 (2.9)	
	Normal	201	129 (64.2)	58 (28.9)	14 (7.0)	
	Above	1	1 (100)	0 (0.0)	0 (0.0)	

deficiency rate, emphasizing the critical role of sunlight in vitamin D synthesis. Additionally, maternal serum calcium (p-value < 0.001) and maternal serum alkaline phosphatase (p-value =0.050) highlighted biochemical factors that significantly affect vitamin D levels, with calcium showing nearly universal deficiency among those with inadequate levels, and

Table 4 Factors Affecting Cord Vitamin D Levels

Factors Affecting Cord Blood Vitamin D Level		Total (n=300)	Cord Blood Vitamin D Level			p-value
			Deficient (n=236) (%)	Insufficient (n=56) (%)	Sufficient (n=18) (%)	
Religion	Hindu	266	205 (77.1)	53 (19.9)	8 (3)	0.151
	Muslim	34	31 (91.2)	3 (8.8)	0 (0.0)	
Area of residence	Rural	157	130 (82.8)	21 (13.4)	6 (3.8)	0.026
	Urban	143	106 (74.1)	35 (24.5)	2 (1.4)	
Maternal education	Primary	19	17 (89.5)	2 (10.5)	0 (0.0)	0.003
	Secondary	61	46 (75.4)	11 (18)	4 (6.6)	
	Senior Secondary	85	66 (77.6)	15 (17.6)	4 (4.7)	
	Graduate	101	73 (72.3)	28 (27.7)	0 (0.0)	
	Above	34	34 (100)	0 (0.0)	0 (0.0)	
Socio economic status of the family	Lower	2	2 (100)	0 (0.0)	0 (0.0)	0.597
	Upper Lower	82	62 (75.6)	18 (22)	2 (2.4)	
	Lower Middle	86	63 (73.3)	19 (22.1)	4 (4.7)	
	Upper Middle	124	103 (83.1)	19 (15.3)	2 (1.6)	
	Upper	6	6 (100)	0 (0.0)	0 (0.0)	
Maternal gravida	Primigravida	111	80 (72.1)	29 (26.1)	2 (1.8)	0.035
	Multigravida	189	156 (82.5)	27 (14.3)	6 (3.2)	
Maternal parity	Nulliparous	238	186 (78.2)	46 (19.3)	6 (2.5)	0.819
	Multiparous	62	50 (80.6)	10 (16.1)	2 (3.2)	
Maternal dietary habits	Vegetarian	114	89 (78.1)	19 (16.7)	6 (5.3)	0.080
	Non-Vegetarian	186	147 (79)	37 (19.9)	2 (1.1)	
Maternal exposure to sun	Inadequate	117	117 (100)	0 (0.0)	0 (0.0)	<0.001
	Adequate	183	119 (65)	56 (30.6)	8 (4.4)	
Gender of neonate	Male	165	131 (79.4)	28 (17)	6 (3.4)	0.390
	Female	135	105 (77.8)	28 (20.7)	2 (1.5)	
Gestational age	Pre-Term	79	58 (73.4)	17 (21.4)	4 (5.1)	0.046
	Term	159	128 (84.2)	20 (13.2)	4 (2.6)	
	Post-Term	38	30 (78.9)	18 (21.1)	0 (0.0)	
	Post Dated	31	20 (64.5)	11 (35.5)	0 (0.0)	

Table 4 Continued

Factors Affecting Cord Blood Vitamin D Level		Total (n=300)	Cord Blood Vitamin D Level			p-value
			Deficient (n=236) (%)	Insufficient (n=56) (%)	Sufficient (n=18) (%)	
Birth weight	Very Low	29	21 (72.4)	6 (20.7)	2 (6.9)	0.602
	Low	69	58 (84.1)	9 (13)	2 (2.9)	
	Normal	201	156 (77.6)	41 (20.4)	4 (2)	
	Above	1	1 (100)	0 (0.0)	0 (0.0)	
Maternal serum calcium	Inadequate	78	75 (96.2)	3 (3.8)	0 (0.0)	<0.001
	Normal	222	161 (72.5)	53 (23.9)	8 (3.6)	
Maternal serum alkaline phosphatase	Normal	21	19 (61.9)	8 (38.1)	0 (0.0)	0.050
	Excess	279	223 (79.9)	48 (17.2)	8 (2.9)	

alkaline phosphatase levels correlating with higher deficiencies, possibly due to their roles in metabolic health (Table 4).

Cord blood vitamin D levels were shown to be significantly associated with vitamin D levels in maternal blood. Among 300 maternal blood samples, only 18 were found to have sufficient vitamin D levels (>30 ng/mL). Of the babies born to mothers in the deficient group, 98.5% were found to be deficient in vitamin D levels. In contrast, none of the neonates born to mothers with sufficient vitamin D levels were found to be deficient. The correlation between maternal vitamin D levels and cord blood vitamin D levels was found to be statistically highly significant (p<0.001) (Table 5).

Discussion

Vitamin D deficiency in pregnant women is considered a major global health problem, and despite abundant sunlight, its prevalence is high in Asian countries, including India. Many recent studies have concluded that low levels of vitamin D in maternal serum depend on a number of factors, including climate, culture, and dietary habits. Numerous studies document that newborns receive their vitamin D entirely from their mothers' vitamin D stores. Both low maternal and cord blood vitamin D levels have been linked to adverse outcomes in pregnant mothers and neonates. Therefore, the importance of early detection and correction of low maternal vitamin D levels may lead to improvements in maternal and child health indices.[10]

In this study, religion was found to significantly correlate with vitamin D levels, with Hindu mothers exhibiting higher sufficiency rates compared to Muslim mothers, who had none. Additionally, maternal gravida status played a notable role; primigravida

Table 5 Correlation Between Maternal and Cord Vitamin D Levels

Maternal Vitamin D	Total (n=300)	Cord Blood Vitamin D Level			p-value
		Deficient	Insufficient	Sufficient	
		Frequency (n=236) (%)	Frequency (n=56) (%)	Frequency (n=18) (%)	
Deficient	196	193 (98.5)	3 (1.5)	0 (0.0)	<0.001
Insufficient	86	43 (50)	43 (50)	0 (0.0)	
Sufficient	18	0 (0.0)	10 (55.6)	8 (44.4)	

mothers had higher vitamin D sufficiency than multigravida mothers. Other factors, such as area of residence, maternal education, socioeconomic status, maternal parity, dietary habits, gestational age, and birth weight, did not show a significant impact on vitamin D levels. Notably, maternal exposure to sunlight was strongly correlated; inadequate sun exposure was associated with a high deficiency rate. While this study provides significant insights into the correlation between maternal and neonatal vitamin D levels, it is important to acknowledge additional factors that may contribute to vitamin D deficiency. Factors such as maternal BMI, physical activity, skin pigmentation, and seasonality are crucial determinants of vitamin D status. Higher BMI has been associated with lower vitamin D levels due to the sequestration of the vitamin in adipose tissue. Similarly, limited physical activity, darker skin pigmentation, and lack of seasonal sunlight exposure are known to affect the synthesis of vitamin D.[11]

Aji conducted a study to assess the serum levels of 25-hydroxyvitamin D (25(OH)D) in the first trimester and its associated factors, including socio-demographics, pregnancy profiles, dietary intake, and maternal anthropometric measurements.[12] The study identified significant independent predictors such as being unemployed, having nulliparous parity, engaging in less than an hour of outdoor activity daily, and not taking supplements prior to pregnancy. Similar observations were reported by authors such as Brian-D Adinma *et al*.[13] and Ates *et al*.[14]

Significant maternal factors affecting cord blood vitamin D levels included area of residence, maternal education, maternal gravida status, gestational age, maternal serum calcium, maternal serum alkaline phosphatase, and maternal sun exposure. In contrast, maternal factors that did not significantly affect vitamin D levels were religion, socioeconomic status, maternal parity, gender of the neonate, and dietary habits. Fink *et al*. undertook a review study to assess factors affecting vitamin D status in infancy, such as the intake of antenatal and postnatal vitamin D supplementation.[15] The review found significant associations between dietary intake, UV exposure, latitude, seasonal variation, and infants' vitamin D status. Although some associations between genetic variation, ethnicity, socioeconomic status, and vitamin D levels have been reported, these were not found to be significantly associated. Similar findings were also reported by authors such as Jamali *et al*[16] and Aletayeb *et al*.[17]

Cord blood vitamin D levels were significantly associated with maternal vitamin D levels; 98.5% of babies born to deficient mothers were also deficient in vitamin D levels. None of the neonates born to mothers with sufficient vitamin D levels were found to be deficient in their vitamin D levels. The correlation between maternal vitamin D levels and cord blood vitamin D was found to be statistically highly significant. Ariyawatkul K *et al*. conducted a study to determine the prevalence of vitamin D deficiency in the cord blood of newborns and its association with maternal vitamin D status.[18] The study found that the mean maternal and cord blood 25OHD levels were 25.42±8.07 ng/mL and 14.85± 5.13 ng/mL, respectively. Vitamin D deficiency (25OHD <12 ng/mL) and insufficiency (25OHD 12-20 ng/mL) in cord blood were observed in 20.2% and 69.1% of newborns, respectively. A significant correlation was found between maternal and cord blood vitamin D levels ($r=0.86$; $p<0.001$). This strong correlation aligns with the findings of similar positive correlations reported by authors such as Wierzejska *et al*.[19] and Treiber *et al*.[20]

The limitations of this study included its cross-sectional nature, the absence of a control group, and a relatively small number of cases. Furthermore, factors that may affect cord vitamin D levels, such as gestational age and weight, were not considered. Randomized controlled trials or comparative studies with a larger number of cases are required to further substantiate the findings of this study. The study demonstrated a strong correlation between maternal and cord blood vitamin D levels, highlighting that deficiencies in mothers are likely to result in deficiencies in neonates. Significant factors influencing vitamin D levels included maternal education, area of residence, gravida status, gestational age, sun exposure, and biochemical indicators like serum calcium and alkaline phosphatase. In contrast, religion, socioeconomic status, maternal parity, dietary habits, and the gender of the neonate were not found to have a significant impact on neonatal vitamin D levels.

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Feasibility, Safety, and Efficacy of Segmental Spinal Anesthesia with Predominantly Isobaric Levobupivacaine: A Tertiary Care Hospital Study

Lalit Goyal,[1] Saloni Gupta,[1] Sachin Nagar,[2] Vinay Bhargava[1]

[1]Anaesthesia and Critical Care, Kota Heart Institute and General Hospital, India
[2]Department of Anaesthesia, Critical Care & Pain, Aarvy Healthcare Super Speciality Hospital, Kalyani Hospital Gurugram, India

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Correspondence:
Lalit Goyal,
Sr. Consultant & Head,
Anaesthesia and Critical Care,
Kota Heart Institute and General Hospital, India
Email: lalitg_ipsa@yahoo.co.in

Abstract

Objective: To assess feasibility, safety, and efficacy of segmental spinal anesthesia with isobaric levobupivacaine in patients undergoing various abdominal and lower limb surgeries.

Methods: This was a prospective cohort study conducted at the Department of Anesthesiology and Critical Care of Kota Heart Institute and group of hospitals in India. The study involved 100 patients undergoing various abdominal and lower limb surgeries under segmental spinal anesthesia (SSA). Isobaric levobupivacaine 0.5% with fentanyl 20–25 µg or dexmedetomidine 5–10 as adjuvant or hypobaric levobupivacaine 0.167% was injected in intervertebral space depending on the surgery. Hemodynamic parameters, postoperative pain level, and adverse effects were analyzed with a p-value less than 0.05 was considered statistically significant.

Results: An overall male predominance was observed with a 1:0.515 male-to-female ratio. The most affected age group was 41–50 years (37%), with a mean age of 44.51±11.72 years. ASA classification indicated 34% of patients in ASA I, 40% in ASA II, 24% ASA III and 2% in ASA IV . The primary surgery was laparoscopic cholecystectomy (26%) in normal risk group and orthopedic in high-risk group(20%). Postoperative pain assessment showed mean VAS scores ranging from 1.92 to 3.42 at different time intervals. Common adverse effects were hemodynamic instability (13%), shoulder tip pain (33%), PONV (3%), headache (6%), pruritis (2%) and urinary retention (1%). The hemodynamic instability showed less incidence in ASA III/IV category.

Conclusion: Segmental spinal anesthesia can be used successfully for abdominal and lower limb orthopedic surgeries in patients of ASA I to ASA IV.

Keywords: ASA grade, levobupivacaine, segmental spinal anesthesia, visual analogue scale

Introduction

Segmental spinal anesthesia (SSA) offers a precise anesthesia for a variety of surgical procedures. In contrast to the conventional spinal anesthesia, the SSA consists of selective administration of local anesthetic agents at a specific spinal level, achieving anesthesia limited only to that particular segment of the body.[1] SSA minimizes venous dilation over a significant portion of the body thereby reducing the intraoperative blood pressure fluctuations.[2] Moreover, the minimal doses of anesthetic drugs are required in SSA due to its targeted action on specific nerve roots, which reduces the risk of systemic side effects in patients undergoing various surgeries. SSA also allows for adequate muscle

relaxation without compromising respiratory or circulatory functions. Segmental spinal anesthesia provides effective anesthesia with minimal hemodynamic changes; therefore, SSA has a favorable safety profile compared to conventional spinal and general anesthesia.[3]

Segmental spinal anesthesia is commonly used in lower limb orthopedic surgeries, abdominal surgeries, various breast surgeries, laparoscopic cholecystectomy, nephrectomies, cystectomy, and lower segment Caesarean sections.[4] Surgeries where SSA can be particularly advantageous include, but are not limited to, inguinal hernia repair and urological surgeries such as transurethral resection of the prostate (TURP). By selectively blocking spinal nerves innervating the operative site while preserving sensation and motor function in other regions, SSA offers the potential for improved intraoperative conditions, reduced postoperative pain, and enhanced patient satisfaction.[5]

One important limitation of segmental spinal anesthesia is that it should not be used as the sole technique in cases where a prolongation of the surgical procedure is expected. Surgeries lasting an extended period require the combined use of spinal-epidural or continuous spinal anesthesia. Moreover, technically, SSA is more demanding, as factors such as the vertebral level for dural puncture, patient positioning, drug dosages, and volume, as well as the levels of sensory and motor blockade, need to be considered carefully. In SSA, the duration of the block is shorter than in conventional spinal anesthesia (the difference is even greater for motor effects than sensory), making it ideal for day-care procedures.[6]

In addition to the type of surgery, patient factors such as pulmonary functions, the risk of complications associated with general anesthesia, etc., are also major considerations for using segmental spinal anesthesia. In patients with chronic respiratory disease, segmental spinal anesthesia (SSA) is helpful because it avoids the use of medication causing impaired mucociliary clearance, avoids mechanical ventilation, and prevents complications that are linked to mechanical ventilation, including the ventilator-associated pneumonia and atelectasis. General anesthesia in these patients may be responsible for ventilator dependence, as well as respiratory complications in the postoperative period. All these complications can be minimized by using segmental spinal anesthesia.[7]

The choice of local anesthetic agent plays a crucial role in the success and safety of SSA. Agents commonly used in SSA are bupivacaine, levobupivacaine, and ropivacaine. Each of these agents have its distinct pharmacokinetic and pharmacodynamic properties.[8] Among them, Levobupivacaine has gained popularity due to its lower cardiotoxicity and similar efficacy compared to racemic bupivacaine. When administered in an isobaric formulation, levobupivacaine offers predictable spread and duration of anesthesia, making it an attractive option for SSA.[9]

Safety can be further enhanced by using a hypobaric formulation or by diluting the drug to reduce its concentration. In patients who are debilitated, have poor muscle mass, or are at high risk due to comorbidities, or in surgeries where motor relaxation is not required and only sensory effects are needed.[10] The safety of SSA in ASA I & II is already established in former studies for routine open and lap surgeries. This study establishes the same safety, efficacy and feasibility for ASA III and IV also.

Methods

This was a prospective cohort study conducted in the Department of Anesthesiology at a Tertiary Care Medical Institute, India. The study duration was from July 2023 to December 2023. A total of 100 patients undergoing various laparoscopic and open abdominal and lower limb surgeries were included based on predefined inclusion and exclusion criteria. The sample size was calculated based on a pilot study on segmental spinal anesthesia, which assumed 90% power and a 95% confidence interval; thus, the required sample size was 90 patients. Consequently, 100 patients who underwent various surgeries under segmental spinal anesthesia during the study period were included. The study participants were patients aged 18 years and older who underwent elective or semi-emergency abdominal and lower limb orthopedic surgeries, provided they gave informed and written consent. Eligible participants were classified under ASA I to IV. Patients under 18 years of age, those who refused consent, and those with contraindications to spinal anesthesia—such as severe thrombocytopenia, bleeding disorders, or local infections at the injection site—were excluded.

A detailed pre-anesthetic evaluation was conducted for all cases. Special attention was given to the cardiac, respiratory, renal, nervous, and endocrine status of patients. Previous anesthetic exposure and drug

sensitivities were documented. A thorough general and systemic examination was performed, and baseline parameters were recorded. An airway assessment was also conducted. Written informed consent was obtained from all patients. Complete blood counts, pre-operative bleeding time, clotting time, blood urea, serum creatinine, blood sugar, TSH, serum electrolytes, ECG, and serological tests for HIV and HBsAg were performed for all cases. Additional investigations were conducted based on patients' history and age. Needed investigations were repeated after the second dose of analgesic postoperatively.

Throughout the surgery, all patients received oxygen via Venturi mask, with an FiO2 ranging from 28% to 40%. An additional 500 mL of colloid solution was administered to patients with good cardiac reserve. Standard monitoring procedures were implemented for both hemodynamic and clinical parameters, as well as the extent of sensory numbness. In cases where a drop in mean arterial blood pressure of over 20% from the initial pre-anesthetic value was observed (hypotension), mephenteramine boluses at a dosage of 6 mg were used, whereas atropine 0.6 mg was administered for a significant drop in heart rate.

For laparoscopic surgeries in this study, pneumoperitoneum was established through either open umbilical access or a Veress needle. The intraperitoneal pressure was maintained within the range of 10 to 12 mmHg. After the surgical procedure was completed, patients were transferred to the recovery area, where they underwent monitoring for a minimum of 30 minutes before being moved to the Surgical Unit.

Patients undergoing various surgeries, including laparoscopic cholecystectomy, total laparoscopic hysterectomy, diagnostic laparoscopic hysteroscopy, laparoscopic hernia repair, laparotomy, and lower limb orthopedic surgery, were included in this study. SSA at the thoracic or lumbar area was administered depending on whether the patient was undergoing abdominal or orthopedic surgery. Isobaric levobupivacaine 0.5% with fentanyl (20–25 µg) or dexmedetomidine (5–10 µg) as an adjuvant was injected. The volume of the drug and the intervertebral space were determined based on the type of surgery (Table 2). In cases of laparoscopic cholecystectomy, the T9-T10 or T10-T11 intervertebral spaces were used; for total laparoscopic hysterectomy, the T10-T11 or T11-T12 spaces were utilized; for laparoscopic hernia, the T9-T10 space was used; for laparotomy, T10-T11; and for lower limb orthopedic surgeries, the L2-L3 intervertebral space was selected. The dose of isobaric levobupivacaine was titrated according to the type and duration of surgery. Hypobaric levobupivacaine was also used in some high-risk (ASA III/IV) orthopedic cases, prepared by adding distilled water to isobaric levobupivacaine.

Average duration of surgery was noted in all cases. The need for additional blocks, such as the Transversus abdominis plane (TAP) block or the fascia iliaca compartment block (FICB), was determined based on the patient's build, the duration of surgery, and the concentration of drug used in SSA respectively. Heart rate, respiratory rate, systolic and diastolic blood pressures, mean arterial pressure, and oxygen saturation (SPO_2) were recorded for all cases. Postoperative pain was assessed at 2, 4, 6, 12, and 24 hours after surgery using the Visual Analog scale. Any adverse effects, including nausea, vomiting, headache, urinary retention, pruritus, post-dural puncture headache, and shoulder tip pain, were noted. The need for conversion to general anesthesia was also recorded. Additionally, mean anesthesia time, duration of surgery, and postoperative pain were documented.

Table 1 Patient Characteristics

	Age Group	No of Cases	Percentage
Age Distribution (Mean Age 44.51+/- 11.72)	18–30 years	15	15.0
	31–40 years	17	17.0
	41–50 years	37	37.0
	51–60 years	12	12.0
	Above 60	19	19.0
	Total	100	100.0
ASA Grade	ASA I	34	34.0
	ASA II	40	40.0
	ASA III	24	24.0
	ASA IV	2	2.0

Table 2 Types of Surgeries, Doses of Anesthetic Drugs, Intervertebral Space Used, Additional Blocks and Duration of Surgery

Variable	Number of Cases	Anesthetic Drug and Dose	Intervertebral Space Used	Average Duration of Surgery (Minutes)	Additional Block Given
Laparoscopic cholecystectomy	26	TV*:1.8–2.2 mL Iso Levobupivacaine (1.4–1.8 mL) + fentanyl 20µg	T9-T10 / T10-T11	90.34	No
Hysterectomy/ hysteroscopy	16	TV:2.1–2.3 mL bupivacaine(H) 0.5mL + Iso Levobupivacaine 1.2 mL + fentanyl (20–25 µg) + dexmedetomidine 10µg	T10-T11 / T11-T12	122.48	No
Laparoscopic hernia repair	22	TV:2-2.6 mL Bupivacaine(H)0.5 mL+ (1–1.5 mL + fentanyl 25 µg + dexmedetomidine 10µg	T9-T10	88.12	Transversus abdominis plane (TAP)
Laparotomy	16	TV:2–2.5 mL Iso LVB 1.5–2 mL+fentanyl 25µg	T10-T11	96.68	No
Orthopedic surgeries	20	TV:1.2–1.6 mL Iso/hypo Levobupivacaine (1.2–1.6) mL	L2-L 3	102.28	Fascia iliaca compartment block (FICB)

Notes: Tv: total drug volume

Results

Out of the 100 cases studied, there were 66 (66%) males and 34 (34%) females, indicating a male preponderance with an M:F ratio of 1:0.515. The most commonly affected age group was 41-50 years (37%), followed by those above 60 years (19%) and 31–40 years (17%). The mean age of affected cases was found to be 44.51±11.72 years. An analysis of the patients based on American Society of Anesthesiologists (ASA) classification showed

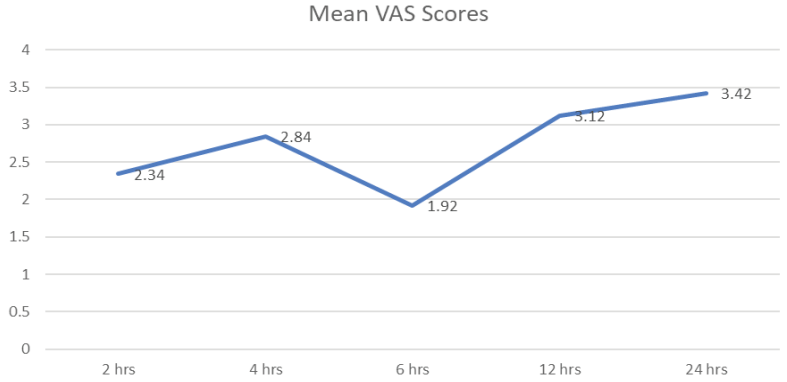

Fig. 1 Mean VAS Scores

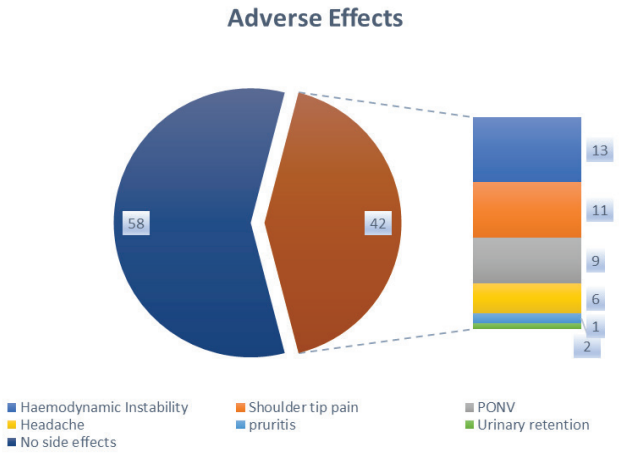

Fig. 2 Adverse Effects

Table 3 Relationship of ASA Grading with Hemodynamic Instability and Conversion into GA

	Number of Patients (%)	Conversion to GA (%)	Hemodynamic Instability (%)
ASA I/II	74	73	77
ASA III/IV	26	27	23

Notes: ASA: American Society of Anesthesiologists; GA: general anesthesia

that 40 (40%) belonged to ASA II, while 34 (34%) belonged to ASA I and 24 (24%) to ASA III. Two (2%) patients belonged to ASA IV (Table 1).

Laparoscopic cholecystectomy was the most common surgery performed, occurring in 26 cases (26%). The remaining surgeries included laparoscopic hernia repair (22%), orthopedic surgeries (20%), total laparoscopic hysterectomy or diagnostic laparoscopic hysteroscopy (16%), and laparotomy (16%). The determination of intervertebral space and dosage of anesthetic drugs were done based on the type and expected duration of surgery. In some cases of laparoscopic hernia repair, a TAP block was administered, while in certain lower limb orthopedic surgeries, FICB blocks was given in addition to segmental spinal anesthesia (Table 2).

Assessment of pain was conducted using Visual Analogue Scale (VAS). VAS scores were recorded at 2, 4, 6, 12, and 24 hours after surgery. The mean VAS score at 2 hours post-surgery was 2.34±0.82, while at 4, 6, 12, and 24 hours, the mean VAS scores were 2.84 ±0.92, 1.92±0.76, 3.12±0.90, and 3.42±1.12, respectively (Fig. 1).

Patients were analyzed for the incidence of adverse effects, which included shoulder tip pain (33%), hemodynamic instability (13%), post-dural puncture headache (PDPH) (6%), postoperative nausea and vomiting (PONV) (3%), pruritus (2%), and retention of urine (1%) (Fig. 2).

There were a total of 11 cases (11%) converted to general anesthesia (GA) for various reasons. It was observed that the incidence of conversion to GA or hemodynamic instability was independent of ASA grading (Table 3).

Discussion

Segmental spinal anesthesia has emerged as a novel technique within the domain of regional anesthesia. It is associated with a rapid onset of anesthesia, precise control over anesthesia levels, reduced systemic side effects, and enhanced safety. These factors are particularly important for patients with comorbidities or compromised respiratory function.[13]

In this study, laparoscopic cholecystectomy was the most common surgery performed on low-risk patients, while lower limb orthopedic procedures were prevalent among high-risk patients. Other surgeries included laparoscopic hernia repair, orthopedic surgeries, total laparoscopic hysterectomy,

diagnostic laparoscopic hysteroscopy, and laparotomy. Many studies have reported the effectiveness and safety of thoracic spinal anesthesia for upper abdominal surgeries, such as cholecystectomy. Singhal *et al*. conducted a study to assess the efficacy and safety of thoracic segmental spinal anesthesia in patients undergoing laparoscopic cholecystectomy.[14] For this purpose, the authors included 50 patients classified as ASA I, II, and III who underwent laparoscopic cholecystectomy under segmental spinal anesthesia. The study found that segmental spinal anesthesia provided complete surgical anesthesia in 48 patients; in two patients, there was a failure to achieve adequate sensory block, necessitating the administration of GA. The median time for full sensory regression was 90 minutes, and the median time for complete motor regression was 60 minutes. No major intraoperative or postoperative adverse events were noted. Based on these findings, the authors concluded that segmental spinal anesthesia offers safe and satisfactory operating conditions for elective laparoscopic cholecystectomy. Similar efficacy of segmental anesthesia for various surgeries has also been reported by other authors, such as Kejriwal *et al*.[15] and Wang *et al*.[16]

The analysis of VAS scores showed the results at 2, 4, 6, 12, and 24 hours after surgery. The mean VAS score at 2 hours after surgery was 2.34±0.82, while the mean VAS scores at 4, 6, 12, and 24 hours after surgery were 2.84±0.92, 1.92±0.76, 3.12±0.90, and 3.42±1.12, respectively. Segmental spinal anesthesia provided adequate analgesia for patients undergoing various abdominal and orthopedic surgeries. Vincenzi *et al*. conducted a study to analyze the benefits of segmental thoracic spinal anesthesia (STSA) with hypobaric ropivacaine for laparoscopic cholecystectomy.[17] Hypobaric segmental thoracic spinal anesthesia was performed on nine patients undergoing elective cholecystectomy. The study found that the mean VAS pain scores postoperatively within the first 12 hours after surgery were 3 (±2) and 4 (±2), respectively. The median length of hospital stay was 2 days (range=1–3 days). Similar effective analgesia in patients undergoing various surgeries under segmental spinal anesthesia was also reported by authors such as Paliwal *et al*.[18] and Haloi *et al*.[19]

Finally, an analysis of the adverse effect profile of patients in this study showed that shoulder tip pain was experienced by 33 (33%) patients and hemodynamic instability was observed in 13 (13%) cases. Other side effects included post-dural puncture headache (PDPH) in 6 (6%) patients, postoperative nausea and vomiting (PONV) in 3 (3%), pruritus in 2 (2%), and urinary retention in 1 (1%). Chandra R conducted a feasibility study of thoracic spinal anesthesia for laparoscopic cholecystectomy. The analysis of the side effect profile in that study indicated that the incidence of paresthesia during needle insertion was 5.8%. Hypotension was observed in 18% of patients, bradycardia in 13%, and nausea in 10%, with shoulder tip pain reported in only 6% of patients.[20] The hemodynamic effects noted in the above study were found to be similar to those in this study.

A total of 11 (11%) cases were converted to GA. Out of these 11 cases, 3 patients were converted due to a prolonged duration of surgery, while 7 patients were converted because of shoulder tip/neck pain that caused distress for both the patient and the surgeon. In 1 patient, conversion was necessitated by significant surgical emphysema that resulted in troubled breathing and hemodynamic instability. In this study, shoulder tip pain emerged as the most common reason for conversion to GA, an issue that needs to be addressed. Similar results were found in a study conducted by Vincenzi *et al*.,[17] which demonstrated that shoulder tip pain is an important factor determining the conversion of SSA to GA.

To address this issue, some pioneering researchers in SSA began using low-concentration hypobaric ropivacaine prior to isobaric ropivacaine with an adjuvant. They reported a significantly reduced incidence of shoulder tip pain. Such promising results have opened the door for researchers to explore this technique of using hypobaric ropivacaine to alleviate shoulder tip pain during surgeries under SSA.

The incidence of conversion to GA or hemodynamic instability was independent of ASA grading. There was no increase in incidence in the high-risk category (ASA III/IV); in fact, hemodynamic instability was borderline less in this group. This suggests that SSA is equally safe or potentially more promising for high-risk patients.

This study included 20 orthopedic procedures, with 12 (60%) of these cases classified as very high-risk (ASA III & ASA IV). Hypobaric levobupivacaine (0.167%) was administered at lumbar levels for these patients. No significant hemodynamic instability was observed. Patients were able

to tolerate oral intake two hours after the procedure, which eliminated the need for intravenous fluids in those with poor cardiac reserves. However, the absence of a control group for comparison was a major limitation of this study. A randomized controlled trial would be more appropriate for assessing the effectiveness of SSA.

Segmental spinal anesthesia can be successfully used for both abdominal and lower limb orthopedic surgeries in patients classified as ASA I to ASA IV. It is particularly effective for hemodynamically unstable patients and those with respiratory diseases, where general anesthesia may carry an unacceptably high risk of complications.

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Original Article

Outcome of Minimally Invasive Percutaneous Plate Osteosynthesis Using Locking Compression Plate for Distal Tibial Fractures

Sagar Rampure, Harsh Choraria, Krishna Hanchate, Sujay K. Mahadik, Shrikant Deshpande

Department of Orthopedics, Bharati Vidyapeeth (Deemed to be University) Medical College & Hospital, Sangli Maharashtra, India

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Correspondence:
Sujay K. Mahadik,
Department of Orthopedics, Bharati Vidyapeeth (Deemed to be University) Medical College & Hospital, Sangli Maharashtra, India
Email: dr.mahadiksujay@gmail.com

Abstract

Objectives: To assess the functional and radiological outcomes of the distal tibia fractures managed surgically with a locking compression plate by MIPPO technique.

Methods: This prospective observational study included 34 patients with distal tibial fractures treated at Bharati Vidyapeeth (Deemed to be University) Medical College and Hospital, Sangli, India. After ethics committee approval and informed consent, eligible adults with complex or unfavorable simple, closed fractures were enrolled. Exclusions were patients under 18 with pathological fractures or comorbidities affecting functional outcomes. Standard preoperative protocols and imaging were conducted. All patients were surgically managed with a locking compression plate by MIPPO technique. Postoperatively, exercises and staged weight-bearing were implemented. Follow-up assessments at 45, 90, 135, and 180 days evaluated pain, movement, and radiological union using Olerud and Molander score. Statistical analysis utilized paired t-test and Chi-Square test.

Results: Out of 34 patients with distal tibial fractures, 24 (70.6%) were males and 10 (29.4%) were females with a mean age of 41.88 years. Most fractures were 42A1 (35.3%) and 43A2 (32.4%). Post-operative superficial wound infection occurred in 3 (8.8%) and deep infection in 2 (5.9%). Mean union time was 16.62 weeks. Functional outcomes were excellent in 3 (8.8%), good in 22 (64.7%), and fair in 9 (26.5%). At six months, complications included ankle stiffness (29.4%), non-union (20.6%), and malunion (8.8%). No cases had plate or screw breakage.

Conclusion: MIPPO technique allows for good functional outcomes while reducing complications, especially in fractures with poor soft tissue conditions.

Keywords: Distal tibial fractures, functional outcome, locking compression plate, percutaneous plate osteosynthesis

Introduction

Distal tibia fractures are typically caused by high-energy mechanisms such as falls from heights or motor vehicle accidents. They can also result from low-energy trauma, as seen in rotational injuries around the ankle.[1] Since the distal tibia has very little soft tissue cushioning, these fractures are very difficult to manage. The management of distal tibial fractures remains a significant challenge due to limited soft tissue coverage and the high complication rates associated with conventional open reduction and internal fixation techniques. This situation prompts the need for alternative approaches that minimize surgical trauma and improve outcomes. Historically, wound complications have been documented to be above 30% after open reduction and internal fixation (ORIF) of distal tibia and Pilon fractures, with infections

reported to be as high as 30% to 55%.[2] The factors c Contributing factors to complications in the management of distal tibia fractures include a high incidence of open and infected fractures.[3]

One of the more commonly used methods for the fixation of distal tibia fractures is antegrade intramedullary nailing (IMN). Studies have shown that it is a reliable fixation method, though it is associated with significant complications.[3] IMN for distal tibia fractures has been linked to knee morbidity, which has been reported to be as high as 71%. Chronic anterior knee pain is among the most common issues, with prevalence reported as high as 73.2%.[4] Additionally, IMN for distal tibia fractures has been shown to have a significantly increased rate of malalignment compared to plate fixation. Open reduction and internal fixation (ORIF) is another frequently used treatment method for distal tibia fractures. However, the extensive dissection involved with ORIF, particularly with the anterolateral approach, has resulted in increased rates of wound complications, including dehiscence, full-thickness necrosis, and ultimately, infection. These complications are serious, as they may necessitate the use of long-term intravenous antibiotics, multiple visits to the operating room for debridement, and may lead to eventual limb loss.[5]

Minimally Invasive Osteosynthesis (MIO) has been used to manage various types of fractures. This technique involves the use of intramedullary nails and percutaneous screws for fracture fixation. In 1996, Krettek *et al*. developed minimally invasive percutaneous plating osteosynthesis for distal femur fractures using a dynamic condylar screw. In their report, the authors demonstrated that infection rates were lower due to the biological healing of the fractures, and the need for bone grafts was reduced compared to open surgeries. This technique features a small skin incision and minimal trauma to surrounding tissues. In addition, it employs a submuscular plate. The depleting technique also emphasizes minimal invasiveness to avoid damaging the normal architecture and callous formation.[6]

Submuscular plating has seen rapid development due to the invention of internal fixators.[7] In this technique, blood supply is preserved by using locking head screws. Furthermore, MIPO was popularized with the development of LCP with combination holes. These plates can be used either as compression plates or in conjunction with internal fixators.[8]

Minimally Invasive Percutaneous Plate Osteosynthesis (MIPO) using a locking compression plate has emerged as a potential alternative, offering reduced soft tissue trauma and preserving periosteal blood supply. However, while MIPO has been adopted for distal femoral and tibial fractures, comprehensive data on its efficacy specifically for distal tibial fractures remain limited. This study aims to address this gap by assessing the functional and radiological outcomes of distal tibial fractures treated with the MIPO technique, thereby contributing evidence to guide clinical decision-making in the management of these challenging fractures.

Methods

This prospective observational study was conducted on 34 patients with distal tibial fractures who presented to the casualty department and were operated on in the Department of Orthopedics, Bharati Vidyapeeth Deemed to be University, Medical College and Hospital, Sangli, India, during the study period. The institutional ethics committee approved the study, and patients were enrolled after obtaining informed consent. Adult patients with complex fractures of the lower third of the tibia or simple, closed fractures that were unfavorable for interlocking nailing were included in this study. Patients below 18 years of age, those with pathological fractures, or individuals with co-morbidities likely to affect the assessment of functional outcomes (such as significant osteoarthritis of the knee, a history of meniscal injuries, or past cruciate ligament injuries) were excluded from the study, as were those with fractures associated with vascular or neurological injuries

Upon arrival at the casualty department, the patients were examined to assess the extent of their injuries. They were evaluated for the presence of vascular or neurological injuries. Analgesics, antibiotics, and intravenous fluids were administered according to the basic protocol, and tetanus prophylaxis was provided as needed. All routine investigations were performed, and imaging studies, including X-rays (lateral and AP views) and CT scans in selected cases, were conducted. All fractures were classified according to the AO-OTA classification.[9] After resuscitating the patient and stabilizing their hemodynamic status, the patients were taken for surgery.

Patients were positioned in the supine position on a radiolucent table, with the unaffected limb kept in an extended position.

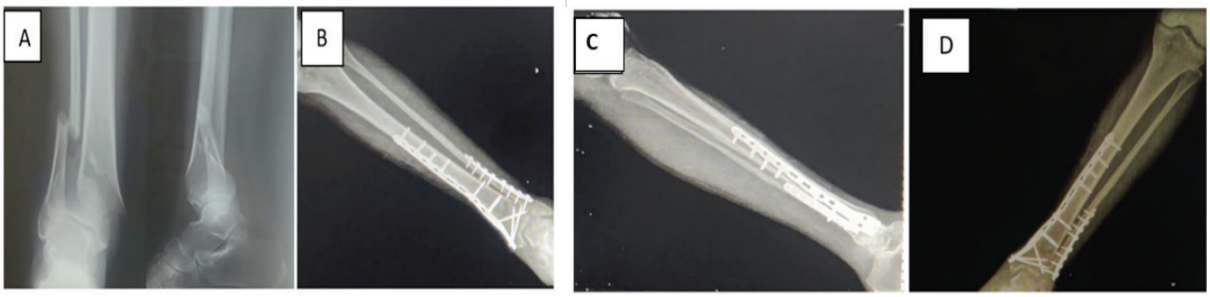

Fig. 1 Preoperative Xray (A), Immediate Postoperative X-rays Anteroposterior and Lateral (B and C) and at the Time of 6 Months Follow Up (D)

A pneumatic tourniquet was applied, and the patient was prepared, leaving the leg exposed for the surgical incision and intraoperative evaluation of the fracture. Intravenous antibiotics were administered prior to inflating the tourniquet. Fracture reduction was performed, and manipulation of the joint was attempted. This was confirmed intraoperatively with the use of a C-arm. The size of the plate was determined based on intraoperative imaging.

An incision was made starting from the medial malleolus and extending proximally over the screw holes to accommodate the plates. While preserving the saphenous vein, a submuscular tunnel was prepared for the plate's insertion using percutaneous elevators. The periosteum of the tibia was exposed by extending the incision longitudinally over the screw holes along the medial side of the tibia, reaching the fracture site. The opposite hand was used to position the plate, and to confirm its location, an allocation method was employed. A K-wire was used to fix the plate to the surface of the tibia. Imaging was then utilized to confirm the correct positioning of the plate. Using the C-arm, it was confirmed that the plate was positioned so that the shaft of the tibia remained central. Fixation screws were inserted following standard procedures. Non-locking screws were initially inserted, while locking screws were inserted after reduction had been achieved. As standard practice, four screws were used for each fragment of the fracture. After the plate fixation, the K-wire was removed, and the screws were secured in place. Wound closure was performed in layers after irrigating the surgical site with normal saline.

Postoperatively, an above-knee splint was maintained for two days, and active and passive exercises were initiated to prevent postoperative stiffness and to strengthen the quadriceps. Sutures were removed on postoperative day 12, and non-weight-bearing walking continued for six weeks. Full weight-bearing was permitted by the 13th week.

Follow-up continued for six months, with assessments at 45, 90, 135, and 180 days, during which patients were evaluated for pain, tenderness, and range of motion at the knee and ankle. During follow-up visits, the functional outcome was assessed using the Olerud and Molander score[10] (Fig. 1).

Qualitative variables were presented as numbers and percentages, while quantitative variables were expressed as means and SD.

Results

Out of 34 studied cases, there were 24 (70.6%) males and 10 (29.4%) females, indicating a male preponderance with a male-to-female ratio of 1:0.71. The majority of patients were either 21–40 years old (47.1%) or 41–60 years old (41.7%). The mean age of the studied cases was 41.88±12.37 years. Twelve (35.3%) patients had a sedentary lifestyle, while moderate and heavy workers comprised 19 (55.9%) and 3 (8.8%) respectively. The right side was affected in 18 (52.9%) of the patients, while the left side was affected in the remaining patients (47.1%).

Table 1 Types of Fractures According to AO-OTA Classification

Fracture Type	Frequency (n=34)	Percentage
42A1	12	35.3
43A1	4	11.8
43A2	11	32.4
43A3	7	20.6

Table 2 Post-Operative Course and Union Time

Variable	Number of Cases	Minimum	Maximum	Mean	Std. Deviation
Non-weight bearing (weeks)	34	6	10	6.82	1.11
Partial weight bearing (weeks)	34	12	18	12.88	1.41
Full weight bearing (weeks)	34	16	22	18.35	1.25
Union time (weeks)	34	14	24	16.62	1.84

Table 3 Functional Outcome as Assessed by Olerud and Molander Score

Outcome by OAMS score	Frequency (n=34)	Percentage
91 to 100 (excellent)	3	8.8
61 to 90 (good)	22	64.7
31 to 60 (fair)	9	26.5
0 to 30 (poor)	0	0

Among the 34 cases studied, the most common type was 42A1 fractures, simple fractures of the distal tibia involving the metaphysis without joint extension, accounting for 35.3% of the cases. This was followed by 43A2 fractures, which are partial articular fractures involving part of the joint surface, representing 32.4%. The 43A3 fractures, complete articular fractures with the fracture line extending through the joint surface, constituted 20.6%, while the least frequent were 43A1 fractures, extra-articular fractures where the fracture line did not extend into the joint, making up 11.8% of the cases (Table 1).

In the immediate post-operative period, superficial wound infection was observed in 3 patients (8.8%), and deep wound infection was seen in 2 patients (5.9%). None of the patients had fat embolism, nerve damage, compartment syndrome, or any vascular injury. Non-weight bearing was continued from 6 weeks until 10 weeks post-operatively, with a mean of 6.82±1.11 weeks. Partial weight bearing was initiated at 12 weeks and continued until 18 weeks post-operatively, with a mean of 12.88±1.41 weeks. Full weight bearing was started at 16 weeks and lasted until 22 weeks post-operatively, with a mean of 18.35±1.25 weeks. The mean fracture union time for the patients was 16.62±1.84 weeks, ranging from 14 to 24 weeks (Table 2).

Table 4 Late Complications (6 Months)

Late Complications at 6 Months	Frequency (n=34)	Percentage
Ankle stiffness	10	29.4
Non-union	7	20.6
Mal-union	3	8.8
Infection	2	5.9
Plate exposure	2	5.9
Plate bend/break	0	0
Screw breakage	0	0

The patients were assessed for functional outcomes using the Olerud and Molander score. Among the 34 patients studied, 3 (8.8%) had excellent outcomes, 22 (64.7%) had good outcomes, and the remaining 9 (26.5%) had fair outcomes. None of the patients had a poor outcome according to the OAMS score (Table 3).

At 6 months post-operatively, 10 patients (29.4%) had ankle stiffness, 7 patients (20.6%) had non-union, 3 patients (8.8%) had malunion, and 2 patients each had infection and plate exposure (5.9% each). None of the patients had bending of the plate or screw breakage (Table 4).

Discussion

In the present study, the mean age of the patients was 41.88±12.37 years, ranging from 19 to 65 years, with the most common age groups being 21 to 40 years and 41 to 60 years (47.1% of the patients each). There was a significant male preponderance, with 70.6% of cases being male. In India, the majority of outdoor and strenuous activities are performed by males, which may explain the predominance of male patients in this study. Sreenivas KD *et al*. measured outcomes in patients with distal tibial fractures treated by the MIPO technique.[11] In their study, the average age of the patients was 44 years, and the most common age group involved

was 31 to 40 years. Males were four times as common as females. Similar age and gender distributions were also reported by authors such as Wennergren *et al*.[12]

The right side was affected in 52.9% of the patients. In the study by Gmachowska *et al*., the right side was also more frequently affected in cases of distal tibial fractures.[13] The most common fracture types in the present study were 42A1 (35.3%) and 43A2 (32.4%). Similarly, Onta *et al*. reported 43A1 as the most common fracture type (35.7%).[14] In the study by Ramesh *et al*.,[15] all fractures were classified according to the Ruedi-Allgower classification and the Gustilo-Anderson classification.The authors included all closed fractures and type I and type II compound fractures according to Gustilo-Anderson, regardless of whether they extended intra-articularly.

Distal tibial fractures are difficult to heal due to the limited soft tissue surrounding the distal tibia and relatively poor blood supply to the area. Therefore, it is important that the surgical procedure achieves biomechanical stability without disrupting callus formation. Complications are known to occur frequently in these patients, largely due to the minimal layer of skin and soft tissue around the distal tibia. It has been reported that infections occur in a significant number of patients.[16] These variations in complications are attributed to differences in the extent of soft tissue injury associated with these fractures, as well as variations in patient profiles.

In the study by Shah *et al*.,[17] out of 15 cases, two patients experienced postoperative infections—one superficial and one deep. The authors managed superficial infections with regular dressings and both topical and parenteral antibiotics. One patient, a known diabetic, developed a wound infection during the postoperative period, which was treated with antibiotics, regular dressings, and strict control of blood sugar levels.

During follow-up, non-weight bearing was maintained from 6 weeks to 10 weeks postoperatively, with a mean duration of 6.82 ± 1.11 weeks. Partial weight bearing was initiated at 12 weeks and continued until 13 weeks postoperatively, with a mean duration of 12.88±1.41 weeks. Full weight bearing commenced at 13 weeks and continued until 22 weeks postoperatively, with a mean duration of 18.35±1.25 weeks. In the study by Jabshetty *et al*., patients were encouraged to ambulate with crutches on the first postoperative day while remaining non-weight bearing. They also began exercises for the foot and ankle.[18] Two months postoperatively, partial weight bearing was initiated, which was subsequently advanced to full weight bearing based on the patient's progress.

In the present study, the mean fracture union time for patients was 16.62±1.84 weeks, ranging from 14 to 24 weeks. Rohit *et al*. conducted a comparative study to evaluate the management of distal tibial fractures treated with interlocking nails and plate osteosynthesis, assessing their functional outcomes using the American Orthopedic Foot and Ankle Society (AOFAS) score and complications. Twenty patients were treated with intramedullary nailing (IMN) and another twenty with minimally invasive plate osteosynthesis (MIPO). The mean union times were 18.45±2.45 weeks for the IMN group and 20±3.21 weeks for the MIPO group. The mean AOFAS scores were 92.6±5.41 for the IMN group and 91.2±6.81 for the MIPO group. The mean union time reported by Rohit *et al*.[19] was similar to that observed in this study.

Among the 34 patients investigated in the present study, 3 (8.8%) had excellent outcomes, 22 (64.7%) had good outcomes, and the remaining 9 (26.5%) had fair outcomes. None of the patients experienced a poor outcome according to the AOFAS score. In the study by Patel *et al*., according to the Teeny and Wiss criteria, 75% of the patients achieved excellent functional outcomes, while another 15% had good functional outcomes. The functional outcome was fair for 10% of the patients, and none had a poor functional outcome.[20]

The limitations of this study include its observational design and relatively small sample size. Additionally, the absence of a control group for comparison may affect the applicability of the findings.

In cases of distal tibial fractures, the MIPO technique significantly reduces surgical tissue trauma while preserving periosteal vascular integrity and the osteogenic fracture hematoma. The good functional results suggest that the MIPO technique should be considered for the surgical management of distal tibial fractures.

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Original Article

Prevalence of Thyroid Function Abnormalities and Its Association with Physical Fatigue in First-Year Medical Students

Arindam Ghosh, Debdeep Dasgupta, Susumna Biswas

Department of Physiology, Bankura Sammilani Medical College and Hospital, Bankura, West Bengal, India

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Correspondence:
Dr. Arindam Ghosh
Assistant Professor,
Department of Physiology,
Bankura Sammilani Medical College and Hospital Bankura
West Bengal, India
Email: arindam01998@gmail.com

Abstract

Objective: To estimate prevalence and type of thyroid function abnormalities and explore their associations with physical fatigue in first-year medical students.

Methods: This was a cross-sectional observational study comprising of 200 first-year medical students. Demographic details and history of any significant medical illness was asked and noted. Thyroid function tests were conducted on all participants. The prevalence and type of thyroid function abnormalities were documented in all students, and the presence and severity of physical fatigue were assessed on the basis of physical fatigue score. Participants with any kind of thyroid function abnormality were compared with those with normal thyroid function tests for presence and severity of fatigue.

Results: Among the 200 students, 144 (72%) were males and 56 (28%) were females, resulting in a male-to-female ratio of 1:0.38. Subclinical hypothyroidism was identified in 9% of students (18 cases), while clinical hypothyroidism was present in 7% (14 cases). Additionally, subclinical hyperthyroidism was detected in 1% of students (2 cases), with no cases of clinical hyperthyroidism reported. Female students were found to be having more thyroid function abnormalities as compared to male students and the difference was found to be statistically significant (p=0.0109). Students with abnormal thyroid function tests (34 cases) had a markedly higher mean FSS score (43.22±4.50) as compared to students with euthyroid (16.85±2.70).

Conclusion: Subclinical thyroid abnormalities are prevalent among young individuals, particularly those with physical fatigue. This study revealed a significant link between presence of fatigue and the possibility of thyroid dysfunction.

Keywords: Physical fatigue, subclinical hypothyroidism, thyroid function abnormalities, thyroid function test

Introduction

The thyroid gland plays an important role in regulating the metabolism, growth, and development. Thyroid hormones (T3 and T4) are synthesized in response to the thyroid-stimulating hormone (TSH) released from the anterior pituitary gland.[1] T3 and T4 influence nearly every cell in the body, primarily by modulating the metabolic rate. They increase oxygen consumption and heat production and play a crucial role in protein synthesis and the metabolism of carbohydrates, lipids, and vitamins. Furthermore, thyroid hormones are essential for normal neurological development, bone growth, and cardiovascular function. The delicate balance maintained by these hormones is crucial for health, and even minor deviations can lead to significant physiological

alterations.[2]

Thyroid function abnormalities are common but often remain undiagnosed, particularly in young individuals, due to the subtle nature of their initial manifestations. Hypothyroidism, hyperthyroidism, and subclinical thyroid dysfunctions are the primary categories of thyroid function abnormalities.[3] Among these, subclinical thyroid dysfunction—characterized by abnormal TSH levels with normal T3 and T4 levels—can be particularly insidious. In young individuals, the clinical presentation of thyroid abnormalities may be minimal or entirely absent in the early stages. However, subtle clinical features such as unexplained physical fatigue, mood disturbances, and cognitive impairment might be early indicators of thyroid dysfunction.[4]

The importance of screening for thyroid function abnormalities in young individuals, particularly those presenting with subtle clinical features such as physical fatigue, cannot be overemphasized. Early detection and intervention are crucial in preventing the progression of subclinical conditions to overt thyroid disease, which can have profound effects on a person's overall health and quality of life.[5] Physical fatigue, although common in the general population, could be an early warning sign of thyroid dysfunction. This is particularly relevant in the context of first-year medical students, in whom the stress of academic workload, combined with the physiological changes associated with early adulthood, can mask the early signs of thyroid dysfunction, making targeted screening even more essential.[6]

Early intervention in young individuals with subclinical thyroid function abnormalities is important. The consequences of a delayed diagnosis can be significant as undetected thyroid function abnormalities may increase the risk of long-term complications, including cardiovascular disease, cognitive decline, and metabolic disorders. Moreover, in a population of first-year medical students, untreated thyroid dysfunction can impair academic performance, increase absenteeism, and contribute to mental health issues such as anxiety and depression. Addressing thyroid dysfunction early in its course allows for timely therapeutic intervention which may prevent the development of more severe health issues.[7]

Despite the established importance of thyroid function in overall health and the potential consequences of undiagnosed thyroid abnormalities there is a significant gap in the literature regarding the prevalence of thyroid function abnormalities in young, asymptomatic populations. Most existing studies focus on middle-aged and older adults, with less emphasis on younger individuals who may not exhibit overt symptoms. This study aims to fill this knowledge gap by investigating the prevalence of thyroid function abnormalities in first-year medical students and examining the association between these abnormalities and physical fatigue.

Methods

This was an observational study conducted in the Department of Physiology at a tertiary care medical institute in India. Two hundred medical students were included in this study based on inclusion and exclusion criteria. Since the study was purely observational, no ethical committee clearance was required. The study included first-year medical students who were above 16 years of age and provided written informed consent. Exclusion criteria included students under 16 years, those who refused consent, those on medications known to affect thyroid function (e.g., lithium, amiodarone, sucralfate, rifampicin), and those with significant psychiatric illnesses. Informed written consent was obtained from all participants. The minimum sample size was calculated based on a pilot study on thyroid functions in asymptomatic young individuals, assuming 90% power and a 95% confidence interval; the required sample size was 180 individuals. Therefore, the researcher included 200 first-year medical students in this study. A detailed history of all participants, including age, gender, and any chronic systemic illnesses such as diabetes, hypertension, or bronchial asthma, was collected. The height and weight of each participant were recorded, and BMI was calculated.

Assessment of all participants for the presence and severity of fatigue was conducted using the Fatigue Severity Scale (FSS), which is a nine-item questionnaire designed to assess the impact of fatigue on a person's daily activities and functioning.[8] Each item is rated on a 7-point Likert scale, where 1 indicates strong disagreement with the statement and 7 indicates strong agreement. The minimum possible score on the FSS is 9 (minimal fatigue), and the maximum possible score is 63 (severe fatigue).

Fasting blood samples were collected in the morning for the determination of thyroid function. The method used for the hormone

estimation was the electrochemiluminescence immunoassay method. The normal reference ranges for T3, T4, and TSH were 0.5-2 ng/mL, 4.5–13.2 µg/dL, and 0.39–4.6 mIU/L, respectively. Based on the thyroid function tests and clinical features, students were diagnosed with either subclinical or clinical hypothyroidism or subclinical or clinical hyperthyroidism. The prevalence and type of thyroid function abnormalities were documented for all students. Participants with any thyroid function abnormalities were compared with those who had normal thyroid function tests regarding the presence and severity of fatigue.

Results

The analysis of gender, age, and Body Mass Index (BMI) among the students revealed that there were 144 (72%) males and 56 (28%) females, resulting in a male-to-female ratio of 1:0.38. In terms of age distribution, a significant majority of the students, 91.00% (182 students), were between 16 and 18 years old, while only 18 (9%) students were in the 19-20 age group. Of the students, 112 (56.00%) had a normal BMI (18.5–24.9), whereas 54 (27.00%) were classified as overweight (25.0–29.9). Additionally, 9 students (4.5%) were underweight (BMI < 18.5), 17 students (8.50%) fell into Obesity Class I (BMI 30.0-34.9), 7 students (3.50%) were classified as Obesity Class II (BMI 35.0–39.9), and 1 student (0.5%) was found to have Class III (BMI > 40) obesity (Table 2).

The analysis of thyroid function status among the students revealed that the majority of the students (83%) were euthyroid. Subclinical hypothyroidism was present in 9.00% (18 students), while clinical hypothyroidism was observed in 7.00% (14 students). Two students (1%) were found to have subclinical hyperthyroidism. No students were diagnosed with clinical hyperthyroidism (Fig. 1).

Among the 34 students with thyroid function abnormalities, there were 16 females (8%) and 18 males (9%). However, considering the gender distribution, a higher percentage of females (16 out of 56, 28.57%) were found to have thyroid function abnormalities compared to males (18 out of 144, 12.50%). Female students exhibited more thyroid function abnormalities than male students, and this difference was statistically significant (p 0.0109) (Table 3).

The analysis of thyroid function tests showed that in the euthyroid group, the mean T3 and T4 levels were 1.78±0.83 and 10.26±3.34, respectively, whereas the mean TSH level was 3.96±2.08. In cases of subclinical hypothyroidism, the mean T3 was 1.47±0.79, the mean T4 was 6.54±2.57, and the mean TSH was 12.62±5.52. For clinical hypothyroidism, the mean T3, T4, and TSH levels were found to be 0.32±0.29, 3.84±2.38, and 37.08±12.54, respectively. In the subclinical hyperthyroidism group, the mean T3, T4, and TSH levels were 0.71±0.08, 5.7±0.4, and 0.2 ±0.01, respectively. No student was found to have clinical hyperthyroidism.

The analysis of thyroid function status in relation to fatigue severity revealed a significant difference between students with normal and abnormal thyroid functions.

Table 1 Fatigue Severity Scale For Assessment of Physical Fatigue

Item	Question	Score
1	My motivation decreases when I am fatigued	1 (strongly disagree) to 7 (strongly agree)
2	Exercise contributes to my fatigue.	
3	I am easily fatigued.	
4	Fatigue interferes with my physical functioning.	
5	Fatigue frequently causes problems for me.	
6	My fatigue prevents me from maintaining sustained physical functioning.	
7	Fatigue interferes with carrying out certain duties and responsibilities.	
8	Fatigue is among my three most disabling symptoms.	
9	Fatigue interferes with my work, family, or social life.	

Table 2 Age, Gender, and Body Mass Index of Participants

Age, Gender and Body Mass Index		Number of Students (n=200)	Percentage
Gender Distribution	Males	144	72
	Females	56	28
	Total	200	100
Age Groups	16–18	182	91
	19–20	18	9
	Total	200	100
Body Mass Index	<18.5 (Underweight)	9	4.50
	18.5–24.9 (Normal)	112	56
	25.0–29.9 (Overweight)	54	27
	30.0–34.9 (Obesity Class I)	17	8.50
	35.0–39.0 (Obesity Class II)	7	3.50
	>40 (Obesity III)	1	0.50

Table 3 Thyroid Function Status of Participants

	Male		Female	
	Number of Cases (n=144)	Percentage	Number of Cases (n=56)	Percentage
Euthyroid	126	63	40	20
Subclinical Hypothyroidism	7	3.50	11	5.50
Clinical Hypothyroidism	10	5	4	2
Subclinical Hyperthyroidism	1	0.50	1	0.50
Hyperthyroidism	0	0	0	0
p= 0.0109 (significant)*				

*Chi-square test

Among euthyroid students, who constituted 83% (166 cases), the mean Fatigue Severity Scale (FSS) score was 16.85±2.70. In contrast, students with abnormal thyroid function tests (34 cases) had a markedly higher mean FSS score of 43.22±4.50. This difference was statistically highly significant, with a p-value of less than 0.0001 and a 95% confidence interval ranging from 25.22 to 27.51, indicating a strong association between abnormal thyroid

Table 4 Mean T3, T4, and TSH Levels

Thyroid Function Tests	Mean T3 (ng/mL)	Mean T4 (µg/dL)	Mean TSH (mIU/liter)
Euthyroid	1.78±0.83	10.26±3.34	3.96±2.08
Subclinical Hypothyroidism	1.47±0.79	6.54±2.57	12.62±5.52
Clinical Hypothyroidism	0.32±0.29	3.84± 2.38	37 .08±12.54
Subclinical Hyperthyroidism	0.71±0.08	5.70±0.4	0.20±0.01
Hyperthyroidism	-	-	-

Table 5 Comparison of Fatigue Severity Scale in Euthyroid and Students with Thyroid Function Abnormalities

Thyroid Status of Students	Number of Students	Mean Fatigue Severity Scale
Euthyroid students	166 (83%)	16.85±2.70
Students with abnormal thyroid function tests	34 (17%)	43.22±4.50
p<0.0001 (Highly significant)	95% CI - 25.22 to 27.51	

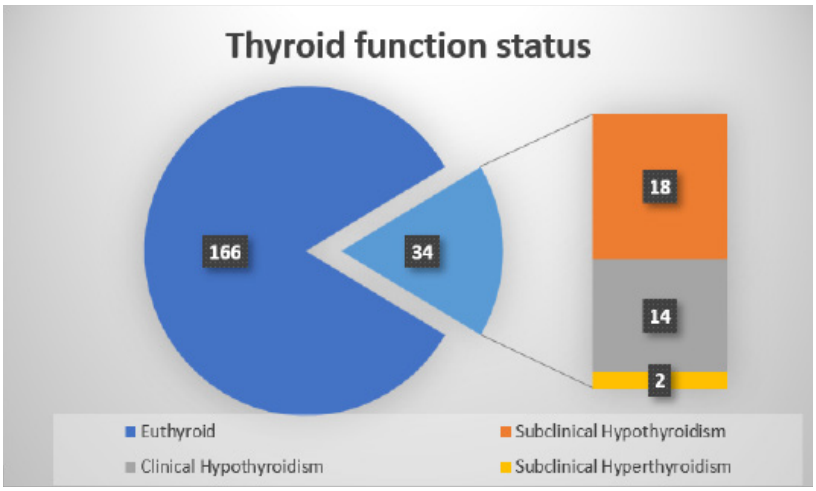

Fig. 1 Thyroid Function Abnormalities

function and increased fatigue severity (Table 5).

Discussion

Thyroid abnormalities are not uncommon in young, seemingly healthy individuals. Subclinical forms of thyroid dysfunction, characterized by biochemical abnormalities without clinical symptoms, are particularly prevalent and can be easily overlooked.[9] These conditions can influence various physiological processes, leading to symptoms such as fatigue, weight gain or loss, and mood disturbances.[10] These signs and symptoms are often ignored by young individuals. The thyroid gland plays a critical role in regulating metabolism and overall homeostasis, making even mild dysfunction potentially significant in affecting an individual's quality of life.[11] In young, healthy individuals, undiagnosed or subclinical thyroid abnormalities can exert insidious but definite effects on physical and cognitive performance.

The majority of the students (83%) were euthyroid. Subclinical hypothyroidism was present in 18 students (9%), while clinical hypothyroidism was observed in 14 students (7%). Two students (4%) were found to have subclinical hyperthyroidism, and no students were diagnosed with overt hyperthyroidism. In a similar study, Iqbal *et al*. assessed thyroid dysfunction and related comorbidities in university students.[12] For this purpose, the authors screened 1,032 students during a two-day medical camp using a questionnaire-based tool. The study found that 7.6% of students had confirmed thyroid disease, with females (10.1%) and individuals aged 15-20 (9.3%) showing higher prevalence rates. Additionally, 23.9% of the participants were identified as high-risk for thyroid dysfunction. Based on these findings, the authors concluded that this screening method effectively identified students at risk for thyroid dysfunction. A similar prevalence of thyroid function abnormalities in young individuals was also reported by authors such as Wanjari *et al*[13] and Delshad *et al*.[14]

Though there were overall more males

with thyroid abnormalities compared to female students, the gender distribution revealed that a higher percentage of females (16 out of 56, 28.57%) had thyroid function abnormalities compared to males (18 out of 144, 12.50%). Unnikrishnan *et al*. conducted a cross-sectional study to investigate the prevalence of hypothyroidism among 5,376 adults across eight cities in India.[15] The overall prevalence was found to be 10.95%, with a striking gender difference. Hypothyroidism affected 15.86% of women, compared to just 5.02% of men, underscoring a significant female predisposition. This disparity was even more pronounced among older women, where the prevalence exceeded 20%. The study also highlighted that 8.02% of the population had subclinical hypothyroidism, with a higher incidence in women. Moreover, 21.85% of participants tested positive for anti-TPO antibodies, further indicating a higher prevalence of autoimmune thyroid disorders in women. These findings suggest that women are more susceptible to hypothyroidism, pointing to a need for increased awareness and targeted screening in this demographic. Similar female preponderance in cases of thyroid disorders was also reported by authors such as Meng *et al.*[16] and Olmos *et al*.[17]

Analysis of the mean fatigue score among euthyroid students showed that the mean Fatigue Severity Scale (FSS) score was 16.85 ± 2.70. In contrast, students with abnormal thyroid function tests (34 cases) had a markedly higher mean FSS score of 43.2± 4.50. This difference was statistically highly significant ($p<0.0001$). Fischer *et al*. conducted a cross-sectional study to investigate the relationship between the hypothalamic-pituitary-thyroid (HPT) axis functioning, fatigue, and early life adversity in women.[18] The study found that lower TSH and higher T4 levels were associated with increased general and physical fatigue, and lower TSH was linked to higher early life adversity. The authors concluded that altered HPT functioning may contribute to fatigue in these patients. Similar correlations between thyroid function abnormalities and physical fatigue have also been reported by authors such as Ruíz-Pacheco *et al*[19]. and Stuber *et al*.[20]

Subclinical thyroid abnormalities are common in young individuals, particularly those who have complaints of generalized fatigue. This study found that students with thyroid function abnormalities had significantly higher Fatigue Severity Scale (FSS) scores compared to those with normal thyroid function. This significant association suggests that fatigue in young individuals may be an indicator of underlying thyroid dysfunction, warranting further clinical evaluation.

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Original Article

Role of Ultrasound Imaging in Children with Dengue Fever: A Retrospective Study

Juliet Anitha Sean Martin,[1] Karthikeyan Annadurai[2]

[1]Consultant Paediatrician, SJ Clinic, Mettupalayam, Tamil Nadu, India
[2]Consultant Pediatrician, Nalam Child Centre, Chennai, Tamil Nadu, India

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Correspondence:
Karthikeyan Annadurai, Consultant Pediatrician, Nalam Child Centre, Chennai, Tamil Nadu, India
Email: Kannadurai19@gmail.com

Abstract

Objective: To study ultrasound findings of children with dengue and analyze the possible correlation between ultrasound findings and severity of dengue in children.

Methods: This retrospective study, which was conducted over one year, analyzed 50 pediatric dengue patients. Ultrasound findings such as gallbladder wall edema, pleural effusion and ascites were assessed. Cases were categorized into dengue without warning signs, dengue with warning signs, and severe dengue according to the World Health Organization's classification of dengue. Correlation between ultrasound abnormalities, severity of dengue, and thrombocytopenia were analyses. A p-value of less than 0.05 was taken as statistically significant.

Results: Of 50 pediatric patients diagnosed with dengue participated in this study, 72% were boys, and 28% were girls with a male-to-female ratio of 1:0.38. Fever was the most frequent symptom (100%), followed by nausea/vomiting (84%) and body ache/myalgia (78%). Ultrasound findings revealed that gallbladder wall edema (48%), pleural effusion (44%), and ascites (50%) were significantly associated with severe dengue and dengue with warning signs ($p<0.001$). Thrombocytopenia was documented in 58% of cases and correlated significantly with ultrasound abnormalities such as gallbladder wall edema, ascites, and pleural effusion ($p=0.0017$).

Conclusion: Ultrasound findings, such as gallbladder wall edema, pleural effusion and ascites, are significantly associated with severe dengue and dengue with warning signs. These features aid in early identification of high-risk patients.

Keywords: Ascites, dengue, gall bladder wall edema, pleural effusion, ultrasound

Introduction

Dengue fever is a mosquito-borne viral infection that poses a significant public health challenge in tropical and subtropical countries worldwide. The disease is caused by the dengue virus, which belongs to the Flaviviridae family and has four distinct serotypes DENV-1, DENV-2, DENV-3, and DENV-4. Transmission of dengue occurs primarily through the bites of Aedes mosquitoes, particularly Aedes aegypti and Aedes albopictus. Over the past few decades, the incidence of dengue has increased dramatically, with the World Health Organization estimating approximately 390 million infections annually.[1]

The burden of dengue is especially high in tropical and subtropical regions such as Southeast Asia, the Pacific Islands, the Americas, and Africa. In India, dengue has become endemic, with frequent outbreaks causing significant morbidity and mortality. The country contributes a substantial proportion of the global dengue burden with

millions of cases reported each year. Factors such as rapid urbanization, increased travel and climate change have further exacerbated the spread of dengue, underscoring the need for ongoing surveillance and effective public health interventions. In pediatric populations, dengue fever presents unique challenges due to differences in immune response and clinical manifestations compared to adults. Children are often at a higher risk of severe symptoms and complications.[2]

The World Health Organization's 2009 classification divides dengue into three categories: dengue without warning signs, dengue with warning signs and severe dengue. This categorization is crucial for clinical management as early identification of warning signs (severe abdominal pain, persistent vomiting, fluid accumulation, mucosal bleeding, lethargy, restlessness, hepatomegaly, and rise in hematocrit and rapid decline in platelet count) is crucial in preventing progression to more severe forms of the disease. Severe dengue, previously known as dengue Hemorrhagic fever and dengue shock syndrome is characterized by plasma leakage, severe bleeding and organ dysfunction requiring prompt and intensive medical care.[3]

The clinical presentation of dengue in children can vary widely, ranging from mild febrile illness to severe, life-threatening conditions. In mild cases, symptoms such as high-grade fever, headache, retro-orbital pain, myalgia, arthralgia and a maculopapular rash are common. As the disease progresses to a more severe form, children may exhibit warning signs that signal a higher risk for severe outcomes including hemorrhagic manifestations, thrombocytopenia and plasma leakage leading to shock. Severe dengue characterized by significant plasma leakage, bleeding and organ dysfunction is a major cause of pediatric hospital admissions and can cause significant morbidity and even mortality if not promptly managed.[4]

The diagnostics for children suspected of having dengue fever includes a thorough clinical evaluation and laboratory investigations. The assessment of hematological profiles is vital for determining disease severity and guiding treatment decisions. Typical findings in dengue include leukopenia, thrombocytopenia, and elevated hematocrit levels, which reflect the degree of plasma leakage. Additionally, biochemical tests may show elevated liver enzymes, indicating hepatic involvement, a common finding in severe cases. Ultrasonography (USG) plays an important role in the evaluation of children with dengue. USG can identify signs such as gallbladder wall thickening, pleural effusion, and ascites which are critical in assessing the severity of the disease and guiding clinical management. Early and accurate diagnosis is essential for timely intervention and can significantly reduce morbidity and mortality in pediatric patients with dengue.[5]

It has been suggested that the degree of thrombocytopenia, hematocrit levels, as well as the evidence of plasma leakage on ultrasound can help predict the risk of progression to severe dengue and guide clinical decision-making.[6] A rapidly falling platelet count and rising hematocrit are strong indicators of severe disease , warranting closer monitoring and more aggressive management.[7] Imaging findings such as pleural effusion, ascites and gall bladder wall edema indicate vascular leakage and impending shock. Utilizing a combination of clinical, laboratory, and imaging criteria allows for a more comprehensive assessment of disease severity.[8]

Despite the growing body of literature on dengue fever, a significant knowledge gap remains regarding the correlation between laboratory and imaging findings and the clinical severity of dengue in pediatric patients. This study was undertaken to analyze the ultrasound findings of children with dengue and to determine whether there is a correlation between various ultrasound findings and the severity of dengue in children.

Methods

This was a retrospective study conducted in the Department of Pediatrics at a tertiary care medical institute located in an urban area, specifically SJ Clinic, Mettupalayam, Tamil Nadu, India. The study duration was one year, extending from January 2023 to December 2023. The sample size was calculated using the formula $n=(Z\ \alpha 2)\ X\ \sigma 2/d2$, based on pilot studies examining ultrasound findings in dengue hemorrhagic fever (Thulkar *et al*.[11]). Assuming 90% power and a 95% confidence interval, the required sample size was 40 patients; therefore, 50 patients were included in this study. The study included pediatric patients under 18 years of age who were diagnosed with dengue based on a positive NS1 antigen, IgM antibody, or RT-PCR test, and whose complete electronic medical records were available. Patients were excluded if they were over 18 years of age, had

incomplete electronic medical records, or if an ultrasound had not been performed. Since this was a purely retrospective observational study and the confidentiality of participants was strictly maintained, no ethical committee clearance was required. It was ensured that all data used in this study was anonymized and that no identifiable information was included in the analysis.

Pediatric patients diagnosed with dengue fever (positive NS1 antigen, IgM antibody, or RT-PCR) during the study period were included based on predefined inclusion and exclusion criteria. Data was extracted from electronic medical records. All pediatric patients with either NS1 antigen or IgM positive for dengue serological markers were included. Cases were categorized into dengue without warning signs, dengue with warning signs, and severe dengue according to the World Health Organization's classification of dengue (Table 1).[9]

Detailed information was obtained from the medical records regarding the duration of illness and the presence of warning symptoms, such as severe abdominal pain or restlessness. Clinical data, including pulse rate, respiratory rate, and blood pressure, were recorded from the patients' charts. Laboratory results, such as serological markers (IgG, IgM, and NS1 or RT PCR), serum electrolytes, complete blood count, hepatic and renal function tests, and coagulation profiles, were also extracted from the electronic medical records. Records of abdominal ultrasounds performed during the patients' hospital stay were reviewed, focusing on findings such as gallbladder wall thickening, fluid collection in the gallbladder fossa, and the presence of pleural effusion and ascites, as reported by the radiologist.

A correlation between ultrasound findings and the type of dengue (dengue without warning signs, dengue with warning signs, and severe dengue) was established. Statistical analysis was conducted using SPSS 22.0 software, and for statistical purposes, a p-value of less than 0.05 was considered statistically significant.

Results

In this study, out of 50 pediatric patients diagnosed with dengue, 36 (72%) were boys and the remaining 14 (28%) were girls. There

Table 1 Dengue Severity Classification According to the World Health Organization

Category	Criteria
Dengue without Warning Signs	Fever and two of the following: • Nausea, vomiting • Rash • Aches and pains • Positive tourniquet test • Leukopenia Laboratory-confirmed dengue (important when no sign of plasma leakage)
Dengue with Warning Signs	Dengue as defined above with any of the following warning signs: • Abdominal pain or tenderness • Persistent vomiting Clinical fluid accumulation (ascites, pleural effusion) • Mucosal bleed • Lethargy or restlessness • Liver enlargement >2 cm Laboratory findings : increase in hematocrit concurrent with rapid decrease in platelet count
Severe Dengue	Dengue with at least one of the following: Severe plasma leakage leading to shock (dengue shock syndrome, DSS) and/or fluid accumulation with respiratory distress • Severe bleeding as evaluated by the clinician Severe organ involvement (e.g., liver AST or ALT >= 1000, CNS impairment, heart and other organ involvement)

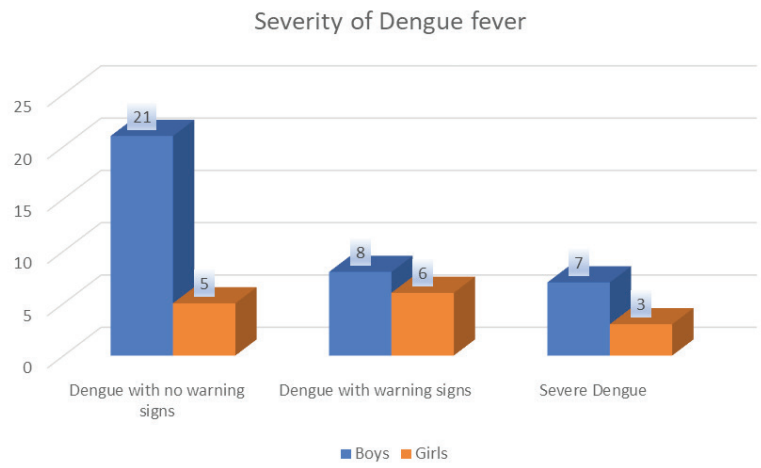

Fig. 1 Classification of Dengue on the Basis of Severity

was a male preponderance with a M:F ratio of 1:0.38. The most commonly affected age group in boys was 11–15 years (20%), whereas among girls, the most commonly affected age group was 5–10 years (14%). The mean age of boys was 10.4±3.4 years, while the mean age of girls was 9.2±2.8 years. The mean ages of boys and girls were found to be comparable in the studied cases (p=0.2467).

Cases were divided into dengue without warning signs, dengue with warning signs, and severe dengue according to the World Health Organization's classification of dengue. There were 21 (42%) boys and 5 (10%) girls who were found to have dengue without warning signs whereas dengue with warning signs was present in 14 (28%) cases and severe dengue was seen in 10 (20%) patients (Fig. 1).

The most common presenting complaint was fever, which was present in all patients (100%). Other complaints included nausea and vomiting (84%), body ache/myalgia (78%), skin rash (62%), conjunctival injection (46%), restlessness (48%), periorbital edema (44%), headache (42%), tachypnea (34%) and hypotension (24%). Less common features included bleeding manifestations (20%), and icterus (6%) (Table 2).

A detailed review of imaging findings showed that 24 (48%) patients had gallbladder wall edema on ultrasound imaging. Other imaging findings indicated that unilateral

Table 2 Signs and Symptoms of Dengue

Sign and Symptom	Number of Cases	Percentage (%)
Fever	50	100
Body ache/myalgia	39	78
Headache	21	42
Nausea/vomiting	42	84
Skin rash	31	62
Conjunctival injection	23	46
Restlessness	24	48
Periorbital edema	22	44
Tachypnoea	17	34
Hypotension	12	24
Icterus	3	6
Bleeding manifestation	10	20

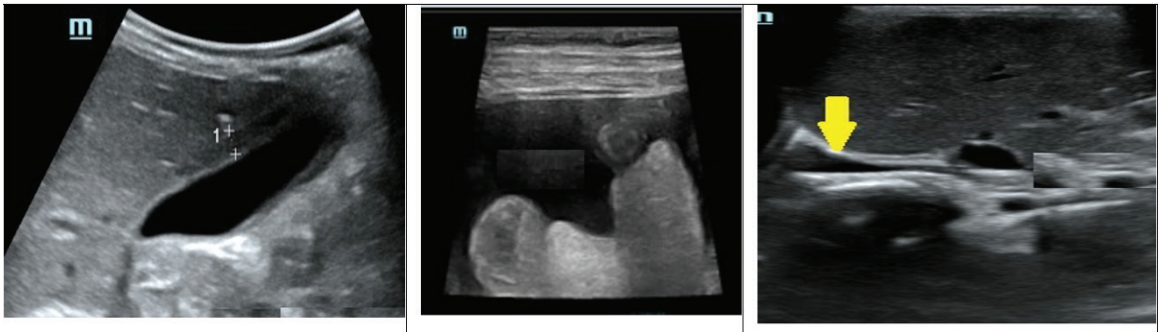

Fig. 2 Common Ultrasound Abnormalities In Patients with Dengue Include Thickened Gall Bladder Wall (Gall Bladder wall edema) (Left), Ascites in the form of Free Fluid in Peritoneal Cavity (Middle) and Right Pleural Effusion (Right)

Table 3 Ultrasound Findings and Their Correlation with Severity of Dengue

USG feature	Total n out of 50 (%)	Non-Severe Dengue (n=26) (%)	Non-Severe Dengue with Warning Signs or Severe Dengue (n=24) (%)	p-value
Pleural effusion	22 (44)	4 (8)	18 (36)	<0.001*
GB Wall edema	24 (48)	5 (10)	19 (38)	<0.001*
Ascites	25 (50)	7 (6)	18 (36)	=0.001*
Hepatomegaly	16 (32)	9 (18)	7 (14)	0.54
Splenomegaly	24 (48)	13 (26)	11 (22)	0.40

*Chi Square test

Table 4 Imaging Abnormalities and Their Correlation with Thrombocytopenia

	Thrombocytopenia Present (platelet count < 1, 50,000/microliter)		Thrombocytopenia Absent platelet count > 1, 50,000/microliter	
	Number of cases	**Percentage**	**Number of cases**	**Percentage**
Gall bladder wall edema, Ascites, pleural Effusion or organomegaly present (1 or more)	21	42	6	12
Gall bladder wall edema, Ascites, pleural Effusion or organomegaly Absent	7	14	16	32
p=0.0017 (Significant)*				

*Chi Square test

or bilateral pleural effusion was present in 22 (44%) patients. Additional ultrasound abdominal findings included ascites (50%), splenomegaly (48%), and hepatomegaly (32%). Ultrasound abnormalities such as gallbladder wall edema, unilateral or bilateral pleural effusion, and ascites were found to be more prevalent in patients with non-severe dengue with warning signs and severe dengue, and the difference was statistically significant. In cases of non-severe dengue with warning signs and severe dengue, organomegaly was more common compared to non-severe dengue; however, the difference was not statistically significant (p=0.068) (Table 4) (Fig. 2).

Out of 28 (56%) patients who had documented thrombocytopenia during the course of their illness, 21 (42%) patients had some imaging abnormality, whereas out of 22 (44%) patients with normal platelet counts, 6 (12%) patients had imaging abnormalities. There was a significant correlation between ultrasound abnormalities such as gallbladder wall edema, ascites, pleural effusion, or organomegaly and thrombocytopenia in cases of dengue in pediatric patients (Table 5).

Discussion

Dengue fever is one of the endemic viral diseases prevalent in developing countries, including India. Poor sanitation, rapid urbanization, increasing construction activities, and water clogging contribute to the breeding of Aedes mosquitoes, which act as vectors for the transmission of dengue fever.[10] Though in many cases, dengue fever remains mildly symptomatic and does not require any treatment other than symptomatic management, in some instances, the disease may advance. In these cases, patients may develop complications, including shock, hemorrhagic manifestations, and multiorgan dysfunction.[11]

Dengue is commonly associated with derangements in hematological parameters, such as rising hematocrit, decreasing platelet count, and elevated hepatic enzyme levels.[12] In addition to laboratory parameters, imaging findings such as gallbladder wall edema, ascites, and pleural effusion are also common, particularly in patients with severe dengue. These imaging findings suggest a strong possibility of plasma leakage, which is a hallmark of severe dengue.[13] Although gallbladder wall edema, pleural effusion, and ascites are not specific findings attributable to dengue fever and can be seen in many other conditions where plasma oncotic pressure is low—such as in cases of hypoalbuminemia secondary to liver diseases or malnutrition and in cases of nephrotic syndrome—their presence in pediatric patients suspected of or diagnosed with dengue may indicate the possibility of progression to severe dengue.[14]

Several studies have reported various ultrasound imaging findings in cases of dengue. V R Santhosh *et al*. conducted a retrospective study to determine the role of ultrasound in diagnosing dengue fever and predicting disease severity by correlating imaging findings with platelet count. For this purpose, the authors studied 96 serologically confirmed dengue patients who underwent ultrasound scanning. The study found that 66.7% had edematous gallbladder wall thickening, 64.5% had ascites, and 50% had pleural effusion. The findings were correlated with lower platelet counts. Based on these findings, the authors concluded that sonographic features like gallbladder wall thickening and pleural effusion are important indicators of dengue fever severity. Similar correlations between abdomen USG findings and the severity of dengue were reported by authors such as Dewan N *et al*[16] and Colbert J *et al*.[17]

In this study, there was a significant correlation between thrombocytopenia and dengue severity. Ultrasound abnormalities such as gallbladder wall edema, ascites, or pleural effusion were found to be significantly associated with non-severe dengue with warning signs and severe dengue, as well as the possibility of thrombocytopenia during the course of illness. Donaldson CD *et al*. conducted a prospective study to assess the utility of ultrasonography in predicting thrombocytopenia and disease severity in dengue patients. For this purpose, the authors undertook a study comprising 176 patients (86 males and 90 females). All patients underwent ultrasound scans upon admission, and disease severity was classified using WHO guidelines. The study found that abnormal ultrasound findings, particularly gallbladder wall thickening, were associated with severe dengue and more pronounced thrombocytopenia from day two of admission. Based on these findings, the authors concluded that admission ultrasonography can predict severe dengue and thrombocytopenia. Similar correlations between thrombocytopenia and ultrasound abnormalities were also reported by authors such as Pothapregada S *et al*[19]. and Sharma *et al*.[20]

One important limitation of this study

was its retrospective nature; a randomized controlled trial would have been a better study design. Moreover, this study did not analyze ultrasound abnormalities and their correlation with outcomes in terms of mortality, which would have been an important factor to consider in cases of dengue in the pediatric age group.

Ultrasound findings such as gallbladder wall edema, pleural effusion, or ascites were associated with severe dengue in pediatric patients. These imaging features were significantly linked to severe dengue and dengue with warning signs, highlighting their value in the early identification of patients at higher risk for complications. Therefore, it can be concluded that routine ultrasound evaluation can be a valuable tool in guiding management and improving outcomes in severe dengue cases.

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Original Article

Nutrition Education Effect in Increasing Knowledge and Ability to Interpret Nutrition Facts in Adolescents

Jovita Octa Meylinda, Ditia Fitri Arinda, Indah Yuliana, Desri Maulina Sari

Nutrition Department, Faculty of Public Health Universitas Sriwijaya, Indonesia

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Correspondence:
Ditia Fitri Arinda,
Nutrition Department,
Faculty of Public Health
Universitas Sriwijaya, Indonesia
Email: ditiafitriarinda@fkm.unsri.ac.id

Abstract

Objective: To evaluate the effectiveness of nutrition education on the understanding and proficiency in interpreting nutrition labels among adolescents aged 16–18 years.

Methods: This quantitative study adopted quasi-experimental and control group designs with a pre-test and post-test approach. The sample size included 60 participants, divided into 2 equal groups, receiving education through lecture method and leaflets, respectively. The intertervention was conducted one day in group 1 and group 2, respectively. Sample selection was conducted using a simple random sampling method. Data were collected through a knowledge questionnaire and details on reading ability were obtained using an interpreting ability checklist through pre-tests and post-tests conducted at the same day. The retrieved data were analyzed using the Wilcoxon Signed Rank Test and the Mann-Whitney U Test (α=0.05).

Results: Nutrition education is shown to enhance the understanding (p-value=0,026) and proficiency of students in interpreting nutrition facts (p-value=0,064).

Conclusion: Nutrition education had a positive impact on the understanding and skill of adolescents in reading nutrition facts. Thus, developing educational materials that can be readily applied in daily life will help consumers in making more nutritionally informed choices when selecting packaged foods.

Keywords: Interpretation ability, knowledge, nutrition education, nutrition facts

Introduction

Adolescence is a crucial phase in human development. This phase is characterized by rapid psychological, physical, and behavioral changes that have a significant impact on overall health. Nutritional issues, such as obesity, being overweight, and underweight, are prevalent among this age group[1] In Indonesia, data pertaining to the nutritional status of adolescents are sourced from the 2018 Basic Health Research. According to the report from the Ministry of Health in 2020, 8.1% of individuals aged 16-18 years had thin or fragile nutritional status, while 13.5% fell into the category of severely overweight or obese.

Furthermore, Palembang, a prominent city in the country, demonstrated a value of 8.69% higher prevalence of overweight among this age group. This figure surpasses the 7.19% average prevalence of overweight in South Sumatra, with obesity being 1.33%, as documented by the Ministry of Health in 2018. Excessive consumption of energy, sugar, salt, and saturated fat represents the primary factors contributing to obesity and non-communicable diseases. In today's modern society, adolescents often gravitate toward packaged foods due to their convenience,

portability, and ease of consumption. It is essential to monitor this dietary preference, as packaged foods tend to be high in sodium, sugar, saturated fatty acids, trans fatty acids, and preservatives.[2] A careful selection of snacks can play a pivotal role in shaping the dietary patterns of adolescents. An effective strategy in this regard includes the incorporation of nutritional information on processed food labels.[3]salt and fat. Factors contributing to the nutrition information label are nutrition knowledge, ability to reading label. The frequency of consumption of instant noodles is seen to determine the relationship of the use of nutrition information label with the frequency of consumption of instant noodles per week. The purpose of this study was to study the relationship between nutritional knowledge, ability to read nutrition information labels, using nutrition information labels on consumers in Jakarta and surrounding areas (Bogor, Depok, Tangerang, and Bekasi This information is designed to offer clear quantitative data about the nutritional content of the food. In addition, it empowers consumers to make more informed choices related to their diet, potentially leading to significant long-term health benefits. The essential message conveyed in the Balanced Nutrition Guidelines emphasizes the importance of becoming accustomed to reading food packaging labels. However, common knowledge often hinders consumers from comprehending and interpreting nutritional information labels, leading to challenges in selecting foods in line with nutritional requirements.[3]salt and fat. Factors contributing to the nutrition information label are nutrition knowledge, ability to reading label. The frequency of consumption of instant noodles is seen to determine the relationship of the use of nutrition information label with the frequency of consumption of instant noodles per week. The purpose of this study was to study the relationship between nutritional knowledge, ability to read nutrition information labels, using nutrition information labels on consumers in Jakarta and surrounding areas (Bogor, Depok, Tangerang, and Bekasi The use of scientific language, particularly in nutritional value information, can pose a significant barrier, necessitating assistance for some consumers in comprehending food labels.[2]

The findings of a study conducted by the National Consumer Protection Agency in 2007 indicated that only 6.7% of consumers in Indonesia paid attention to labels on packaged food products when making their selections, a proportion considered low[4]. In contrast, a similar study conducted in a different year revealed that 36.5% of Indonesians were more inclined to read and pay attention to halal labels, a figure not significantly different from the 34.9% who focused on expiration dates. Furthermore, 20.6% of consumers paid attention to the product name, while only 7.9% considered the composition and nutritional information[5].

Studies have shown that individuals who do not read labels tend to consume higher quantities of fat, cholesterol, and sugar in their diets.[6] The provision of nutritional content information is a crucial educational and practical tool for encouraging healthier food choices. This is because one of the key factors influencing the health of individuals is nutritional understanding. Nutrition education plays an essential role in increasing knowledge and fostering positive attitudes toward selecting foods and snacks.[7] Recognizing the challenges, a study was initiated in the Ilir Barat 1 District of Palembang City. The selected areas were *SMA Negeri 1* and *SMA Negeri 2* Palembang, due to their strategic location within the city. These places were situated near minimarkets and canteens that offer packaged food. Additionally, high school students aged 16–18 represent a significant segment of the adolescent population. Consequently, this study aimed to investigate the impact of nutrition education on the knowledge and proficiency in reading Nutrition Facts among adolescents aged 16–18 in the city of Palembang.

Methods

This study adopted quasi-experimental and control designs with both pretest and posttest assessments. Group 2 served as a basis for comparison and received interventions through leaflets, while Group 1 consisted of adolescents who received interventions comprising food models (packaged foods) accompanied by explanations. The approach adopted was lecture methods with PowerPoint slides and food models. In this study, packaged food was served as an educational medium and was used to assess the reading ability variable. A total of four types of packaged foods, namely packaged drinks, foods containing MSG, sweet foods, and instant foods, were selected as samples. The sample size for this study was determined using the hypothesis testing

formula for comparing the two independent population means. A total of 60 subjects were selected using a random sampling method facilitated by Microsoft Excel. The study samples comprised students from two high schools, namely SMA Negeri 1 (Group 1) and SMA Negeri 2 (Group 2), in Ilir Barat 1 sub-district, Palembang City. Data were collected during the period of May 17th to 19th, 2022. The inclusion criteria were students aged 16-18 years, willingness to participate as respondents, provision of informed consent, and good health. The obtained data comprised demographic information about the respondents, pretest and posttest outcomes related to the students' knowledge of nutrition facts using a questionnaire, and pretest and posttest data assessing reading abilities related to nutrition facts through a reading ability checklist. According to Arikunto, knowledge is divided into three categories, namely good, sufficient, and limited knowledge, where respondents can correctly answer 76-100%, 56-75%, and less than 56% of the total questions, respectively. In the interpreting ability variable, respondents are considered to have good ability if they can answer all the questions in the reading ability checklist correctly, without a single mistake. Validity testing was conducted on the sections of the questionnaire pertaining to knowledge and the ability to read Nutrition Facts. Validity testing employed the corrected item-total correlation method. Validity and reliability tests were conducted on 30 individuals who met the same criteria as the study's target population, namely the students of Senior High School 10 Palembang. The statistical tests employed in this study were the Wilcoxon Signed Rank Test and the Mann-Whitney U Test.

Results

Table 1 presents an analysis of respondent characteristics, comprising age, gender, parental income, and pocket money, within both the experimental and control groups. The majority of respondents in the two groups were 17 years old and female. In group 1 and group 2, respectively, the income of most parents was above and below the Regional Minimum Wage of Palembang City. Additionally, the average student school allowances in group 1 exceeded 15,000 IDR, while in group 2, it averaged 15,000 IDR.

Table 2 presents the analysis of the Knowledge category for both group 1 and group 2. The respondents were assessed and classified under "not good," "sufficient," and "good." In both group 1 and group 2,

Table 1 Respondent Characteristics

Characteristics	Group 1		Group 2	
	Frequency (n=30)	Percentage (%)	Frequency (n=30)	Percentage (%)
Age				
16 years old	9	30.0	8	26.7
17 years old	20	66.7	20	66.7
18 years old	1	3.3	2	6.7
Sex				
Male	11	36.7	13	43.3
Female	19	63.3	17	56.7
Parents' Income				
Above Minimum Regional Wage	26	86.7	13	43.3
Under Minumum Regional Wage	4	13.3	17	56.7
School Allowance				
≥15,000 IDR	21	70.0	15	50.0
<15,000 IDR	9	30.0	15	50.0

Table 2 Categorization of Respondents' Knowledge Before and After Education

Category	Group 1				Group 2			
	Pretest		Posttest		Pretest		Posttest	
	(n=30)	%	(n=30)	%	(n=30)	%	(n=30)	%
Not Good	0	0	0	0	3	10	0	0
Sufficient	22	73.3	6	20	26	86.7	11	36.7
Good	8	26.7	24	80	1	3,3	19	63.3

Table 3 Categorization of Respondents' Interpreting Abilities Before and After Education

Category	Group 1				Group 2			
	Pretest		Posttest		Pretest		Posttest	
	(n=30)	%	(n=30)	%	(n=30)	%	(n=30)	%
Good	6	20	22	73.3	1	3.3	16	53.3
Not Good	24	80	8	26.7	29	96.7	14	46.7

the majority of respondents initially had sufficient knowledge about nutrition facts before receiving education. Subsequently, this variable improved, with the majority attaining a "good" level after the educational intervention.

Table 3 shows the Reading Ability for both group 1 and group 2, classified into "Good" and "Not Good" categories. In both groups, the majority of respondents required improved skills in interpreting nutrition facts, with only a few demonstrating good abilities before the educational intervention. Following the intervention, the number of good abilities increased in both groups. However, group 1 had a more significant number of respondents in this category compared to group 2.

Table 4 presents the results of the Wilcoxon Rank Test statistic in group 1, where food model educational media accompanied by explanations was utilized. A p-value of <0.001 was obtained, signifying the impact of providing nutrition education on the knowledge of the respondents about nutrition facts.

In group 2, using leaflet educational media, a p-value of <0.001 was obtained, indicating the influence of nutrition education on the knowledge of respondents about nutrition facts. Furthermore, based on the results of the Mann-Whitney U Test for the knowledge variable, a p-value of 0.026 was achieved, signifying a significant average difference between group 1 and group 2 following the

Table 4 Bivariate Analysis of Knowledge Variable

Category	Group 1		Group 2	
	Frequency	Percentage	Frequency	Percentage
Wilcoxon Rank Test				
Decreasing score	1	3.3	3	10
Increasing score	24	80	24	80
Stable score	5	16.7	3	10
p-value	0.000		0.000	
Mann Whitney U Test				
Mean rank	35.32		25.68	
p-value	0.026			

Table 5 Bivariate Analysis of Interpreting Ability Variable

Category	Group 1		Group 2	
	Frequency	Percentage	Frequency	Percentage
Wilcoxon Rank Test				
Decreasing Score	2	6.7	2	6,7
Increasing Score	22	73.3	23	76,6
Stable Score	6	20	5	16,7
p-value	0.000		0.000	
Mann Whitney U Test				
Mean rank	34.13		2687	
p-value	0.064			

intervention.

Table 5 displays the results of the Wilcoxon Rank Test statistic in group 1, utilizing food model educational media accompanied by explanations. A p-value of 0.000 was achieved, signifying the impact of providing nutrition education on the reading ability of the respondents related to nutrition facts. In group 2, using leaflet educational media, a p-value of 0.000 was also obtained. However, in the Mann-Whitney U Test for the reading ability variable, a p-value of 0.064 was obtained, indicating that there was no significant average difference between group 1 and group 2.

Discussion

Nutrition education is one of the specific nutritional interventions that can be implemented to observe changes in nutritional knowledge among adolescent groups. Knowledge is one of the factors that stimulate or encourage the implementation of a health behavior.[8] Knowledge, as a predisposing factor, plays a crucial role in shaping initial perceptions of health and illness. Consequently, nutrition education is an important effort to develop knowledge that will subsequently influence individuals' perceptions, attitudes, and behaviors.

In this study, the data used were primary data collected using questionnaires and pre-test and post-test checklists for both group 1 and group 2. Scores were tabulated by assigning 1 point for each correct answer and 0 points for each incorrect answer. Each set of answers was tabulated and used for the analysis of the knowledge and reading ability variables. Intervention was administered to both group 1 and group 2 with the same content to minimize bias in the study. The educational material included information about the definition of nutrition facts, the functions of nutrition facts, the components of nutrition facts, how to read and understand nutrition facts, recommendations for natrium, sugars, and saturated fatty acid consumption limits, and the effects of excessive natrium, sugars and saturated fatty acid consumption.

Parents with higher incomes tend to provide their children with more pocket money which is closely associated with the selection of snack foods.[9] Lack of nutritional knowledge can contribute to errors in food choices and is one of the root causes of nutritional issues. In addition, eating habits during adolescence can have long-term health implications.[10] Understanding how to interpret nutritional information labels is crucial for consumers who need to manage their dietary intake, especially those with an obese nutritional status. These labels facilitate the selection and regulation of food consumption from packaged products. The results of the Wilcoxon Rank Test statistic in the Group 1 indicate that providing nutrition education had a positive effect on the knowledge of the respondents about nutrition facts. These results were in line with the study conducted by Murtiningsih on the impact of health education on the knowledge level of mothers with malnutrition under five in the work area of *Puskesmas Babakan Sari*, Bandung City, in 2018. This study demonstrated the influence of health education on the knowledge of mothers who were dealing with malnutrition.[11]

Additionally, the report by Yulinda and

Nurul indicated that counseling using the lecture method predominantly engages the senses of sight and hearing. Approximately 75% of knowledge is transmitted to the brain through the sense of sight, with 87% and 13% being acquired by sight and hearing, respectively. Only about 12% of knowledge is obtained from other senses. Therefore, education group 1 proved to be effective in enhancing the knowledge of respondents.[12] Based on the results of the Wilcoxon Rank Test statistic in Group 2, which utilized leaflet educational media, it was evident that nutrition education had a significant impact. This is consistent with the study conducted by Pakhri et al., which explored the effects of nutrition education on nutrition knowledge, energy, protein, and iron intake in adolescents using leaflet media. The result showed a p-value of 0.000, signifying the influence of nutrition education on the nutritional knowledge of adolescents.[13]

Delivering information and nutrition education through print media such as leaflets, posters, and pocketbooks is more effective. Pictures and color arrangements complement these static visual messages. In the context of this nutrition education study, group 2, which received education through leaflets, experienced an increase in knowledge both before and after nutrition education.[14] Furthermore, educational methods involving a combination of pictures and words, accompanied by sound explanations, are more effective for memory retention compared to those solely comprised of pictures with words.[15] Proficiency in accurately and precisely reading and interpreting nutrition facts is a skill that all consumers of packaged foods should acquire. This ability can empower the making of healthier food choices tailored to nutritional needs and status. To facilitate this, specific nutrition interventions, such as nutrition education, should be implemented to ensure that packaged food consumers comprehend nutrition labels.

Data on the reading ability of respondents were collected through interviews or direct question-and-answer sessions with the respondents. During these interactions, a packaged food item was provided, and inquiries were made concerning quantitative information listed on the nutrition facts label, including the number of servings, total calories, protein, fat, and the significance of terms such as "*Angka Kecukupan Gizi*" and "2150 kcal." When the respondent can answer each of these inquiries, their reading ability is considered proficient. However, in instances where any indicators are still not met, it is deemed to be adequate.

The results of the Wilcoxon Rank Test statistic in group 1, utilizing food models and PowerPoint (PPT) educational media, showed that nutrition education had a discernible impact on the reading ability of the respondents. This was in line with the study conducted by Kartini et al., which explored the influence of counseling on knowledge and practices related to balanced nutrition among elementary school children. The results indicated an enhancement in the nutritional practices of students following counseling.[16]

The Mann-Whitney U Test was conducted to assess the difference in the average outcomes of knowledge and the ability to read nutrition facts after nutrition education was provided between group 1 and group 2. Furthermore, the test for the knowledge variable yielded a p-value of 0.026, signifying a significant difference in the average outcomes between the 2 groups following the intervention. However, the reading ability variable produced a p-value of 0.064, indicating no significant difference in the average outcomes.

The reading ability of students is influenced by two factors, namely internal factors, such as motivation from within students, as well as external factors, including methods, media, and teachers.[17] In this study, the experimental group received education directly through the use of PowerPoint media and packaged food, while the control group utilized leaflet media. However, students in the control group were permitted to pose questions about the leaflets they were provided, and they responded with explanations. Consequently, there is no significant difference in the reading ability variable between the two groups due to external factors, primarily stemming from the provision of explanations related to the material.

In this study, packaged food was served as an educational medium and was used to assess the reading ability variable. A total of four types of packaged foods, namely packaged drinks, foods containing MSG, sweet foods, and instant foods, were selected as samples. However, it would have been beneficial to conduct a preliminary food survey to identify the products or brands most frequently consumed by teenagers. The selection was limited to foods that were familiar to teenagers. Additionally, the educational leaflet for group 2 did not include a breakdown of the types of fats.

To measure the distance of an intervention based on evaluation theory, approximately 2 weeks is needed for knowledge and attitudes, while behavior requires a minimum of 1 month. This is in line with the concept of the sleeper effect proposed by Bringham, which states that people still remember the content of a message 10–14 days after it is delivered.[18] Therefore, in this study, there was no significant difference in the average reading ability between the group 1 and group 2, as the intervention and evaluation were conducted within a relatively short period.

In summary, nutrition education led to increased knowledge and reading ability in both group 1 and group 2. Furthermore, there was a significant difference in knowledge levels between these groups, but no difference was observed in the reading ability variable. Therefore, it was also recommended to conduct further research on the effects of nutrition education about nutrition label on the practice and attitude towards packaged food consumption and its' effects on adolescents' nutritional status through a series of educations and monitoring and evaluation.

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Original Article

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Differential White Blood Cell Count and COVID-19 Hospital Length of Stay: A Post-hoc Analysis

Gita Fajri Gustya,[1,2] Darrin Ananda,[1,2] Muhammad Ilham Dhiya Rakasiwi,[1,2] Farchan Azzumar,[2] Erlina Burhan[2,3]

[1]Faculty of Medicine University of Indonesia, Jakarta, Indonesia
[2]Respiratory and Tuberculosis Research and Training Center, Jakarta, Indonesia
[3]Department of Pulmonology and Respiratory Medicine, Faculty of Medicine University of Indonesia, Persahabatan Central Hospital, Jakarta, Indonesia

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Correspondence:
Muhammad Ilham Dhiya Rakasiwi, Faculty of Medicine University of Indonesia, Jakarta, Indonesia;
Respiratory and Tuberculosis Research and Training Center, Jakarta, Indonesia
Email:
muhammad.ilham65@ui.ac.id/
m.ilhamdhiya@gmail.com

Abstract

Objective: To explore the association between differential white blood cell count and hospital length of stay (LOS) in COVID-19 patients.

Methods: This study is a post-hoc analysis of two prospective cohort studies involving hospitalized COVID-19 patients who received standard therapy, including antiviral and supportive treatments at Persahabatan Hospital, Jakarta, Indonesia, during the Delta and Omicron dominant pandemic periods. Baseline differential white blood cell count before initiation of therapy were documented. LOS was categorized as ≤10 days and >10 days.

Result: Data from 463 subjects were included with most subjects were males (62.2%) with a median age of 54 (14–93) years. The average LOS for subjects was 12.7 (12.1–13.4) days. Bivariate tests showed that lymphocytes, neutrophils, monocytes, neutrophil-lymphocyte ratio (NLR), and neutrophil-monocyte ratio (NMR) had significant association ($p < 0.05$) to LOS. Logistic regression showed that higher monocyte counts were associated with shorter LOS (adjusted OR 0.89; 95% CI 0.840–0.943; $p<0.001$). ROC curve showed that higher monocyte counts (>8.35×10^3/μL) at admission may predict shorter hospitalization (<10 days).

Conclusion: Monocyte count may serve as a potential marker for length of stay in COVID-19 patients, offering key insights for optimizing patient management and resource allocation.

Keywords: COVID-19, length of stay, monocyte, neutrophil, risk factor

Introduction

The global pandemic of Coronavirus disease 2019 (COVID-19) posed one of the most significant threats to public health worldwide. The rapid transmission characteristics of SARS-CoV-2 led to a surge in patients, significantly increasing the demand for healthcare resources beyond their capacity.[1] Indonesia was one of the countries that experienced a dramatic rise in COVID-19 cases, particularly during the first quarter of 2021, the subsequent third and fourth quarters of 2021, and the first and second quarters of 2022, with various virus variants (i.e., Delta, Omicron).[1,2]

The COVID-19 pandemic resulted in increased demands for screening and testing of suspected cases, contact monitoring, patient isolation, and intensive care unit (ICU) management of severe cases.[2] However, the overwhelming number of Omicron cases led to a significant rise in hospitalizations worldwide. Patients infected with the Omicron variant

were less likely to develop severe disease compared to those who contracted previous variants.[3]Research also suggests that patients admitted to hospitals during the mixed Omicron/Delta variant period had shorter hospital stays than those admitted during the Delta variant period. This finding contradicts the hospitalization rates observed.[4]

Hypoxemia is the most common reason for hospitalization in COVID-19 patients and varies in severity. Mild cases are managed with oxygen therapy, while severe cases may progress to Acute Respiratory Distress Syndrome (ARDS) and require mechanical ventilation. The underlying mechanism of hypoxemia in COVID-19 is thought to involve ventilation/perfusion (V/Q) mismatch due to vascular pathology in the lungs. This mismatch is driven by an intense inflammatory response, which increases lung permeability and disrupts pulmonary perfusion regulation. Additionally, an imbalance in procoagulant and fibrinolytic activity contributes to microthrombi formation, further worsening the vascular pathology.[5–7]the causative agent of coronavirus disease 2019 (COVID-19

A study in India showed that white blood cell differential counts are associated with the severity of COVID-19 infection. Indicators of disease severity include neutrophilia, lymphopenia, monocytopenia, a high neutrophil-lymphocyte ratio (NLR), and a high neutrophil-monocyte ratio (NMR).[8] Several laboratory parameters associated with infection and inflammatory processes have been identified as potential predictors of severity and length of stay (LOS) among patients inflicted with COVID-19.[9,10] This study aimed to investigate the relationship between infection markers, specifically differential white blood cell counts, and the LOS in COVID-19 patients during two dominant pandemic waves in Indonesia.

Methods

Post hoc analysis was conducted on datasets from two previous cohort studies in Jakarta.[11] The first cohort study, the REMISI Study, evaluated the safety and effectiveness of antiviral drugs (Remdesivir and Favipiravir) in COVID-19 patients following Emergency Use Authorization (EUA) in Indonesia during the Delta wave (April-August 2021).[12] The second study, the MEGACRON Study, was a prospective cohort study that compared the effects of antiviral therapy on PCR conversion in hospitalized COVID-19 patients during the Omicron wave in Jakarta (January–February 2022).

The post hoc analysis was conducted at Persahabatan General Hospital in Jakarta from January to March 2024, following the completion of two prior cohort studies. MID and FAR coordinated to obtain permission from the data custodians of each study to conduct additional analyses on the existing datasets. Each dataset from the two previous studies contains masked patient identity data to protect the confidentiality of the subjects, along with clinical baseline data and initial laboratory results collected at the time of hospital admission. GFG, DAN, and MID carried out the data screening and cleaning process.

The workflow of each study is illustrated in Fig. 1. In order to achieve a homogeneous subject population, only adult and adolescent subjects >13 years old confirmed with COVID-19 who were hospitalized and received standard COVID-19 therapy according to national guidelines (antivirals and supportive therapy) were included in the analysis. The exclusion criteria for this analysis included incomplete data (absence of admission or discharge dates) or if the patient had died.

The data collected for this study includes demographic information about the subjects, body mass index, presence of comorbidities, and length of hospital stay. The laboratory data analyzed consists of initial lab results obtained on the first day subjects were admitted to the hospital, before receiving antiviral medication. The initial laboratory results included differential white blood cell counts, which were reviewed by the attending physician and are part of the patient care program (not solely for research purposes). The laboratory automatically calculated the neutrophil-lymphocyte ratio as part of routine infection markers. The lymphocyte-monocyte ratio and neutrophil-monocyte ratio were calculated manually by the researchers (GFG, DAN, and MID) under the supervision of EBN, with consultation from a clinical pathologist.
The treatment duration is calculated from when the patient first entered the emergency department (for non-referred cases) or the isolation room (for referred cases). In this analysis, the researcher categorized the length of stay using a 10-day cut-off, where a stay of 10 days or more is considered prolonged. The selection of the 10-day cut-off is based on literature indicating that the average length of stay for COVID-19 patients ranges from 4 to 10 days,[13,14] and for patients with community-acquired pneumonia, the range is between 5

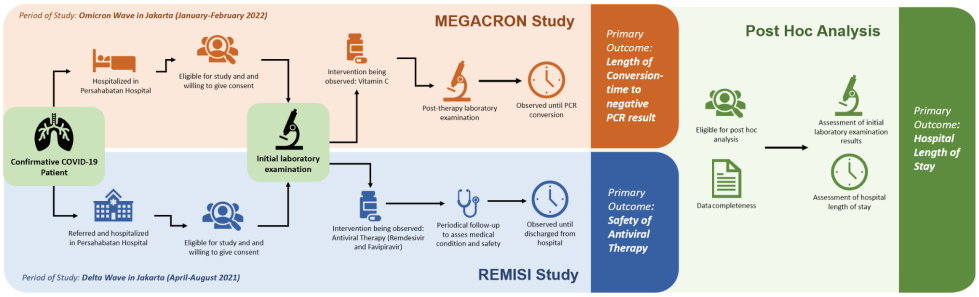

Fig. 1 Subject Recruitment Flow and Post-hoc Analysis

and 9.8 days.[15,16]

In this study, no sample size calculation was conducted; the authors used a convenience sample derived from the available cohort based on specific inclusion criteria. Participant characteristics were summarized using median and interquartile ranges to represent central tendency and variability, particularly for non-normally distributed data. A bivariate analysis was performed to compare leukocyte differential counts between two groups (<10 days and ≥10 days) using an unpaired T-test to identify significant differences. Variables with a p-value <0.25 were further analyzed using logistic regression to explore relationships between multiple variables and treatment duration, adjusting for age, gender, BMI, and comorbidities. The optimal cut-off values for each variable were determined through receiver operating characteristic (ROC) analysis with a 95% confidence interval, which helped assess the sensitivity and specificity of the variables in predicting treatment duration. All statistical analyses were performed using SPSS version 25, with a significance level set at $p<0.05$ to ensure the reliability of the findings.

The ethical review and informed consent process for this post hoc analysis adhered

Table 1 Subject Characteristics

Parameter	Delta wave Period (n = 415)	Omicron wave Period (n = 48)	p-value
Demographic characteristics			
Age [median (IQR), year]	54.5 (20–93)	49.5 (14–75)	<0.001
Gender, n(%) Female Male	 153 (63.1) 262 (36.9)	 22 (45.8) 26 (54.2)	0.225
BMI [median (IQR), kg/m^2]	25.71 (15.56–51.65)	22.63 (16.04–38.57)	<0.001
Presence of comorbidity n (%) Yes No	 374 (90.1) 41 (9.9)	 36 (75) 12 (25)	0.002
Clinical characteristics			
Length of stay (days)	12 (2–47)	7 (1–12)	<0.001
Neutrophil (10^3/µL)	78.95 (10.7–96.2)	69.65 (29–92.3)	<0.001
Lymphocyte (10^3/µL)	13.4 (1.3–86.3)	19.1 (4–58.4)	<0.001
Monocyte (10^3/µL)	7.1 (0.8–22.1)	8.3 (3.5–14.5)	0.002
NLR (%)	5.91 (0.12–73.46)	3.49 (0.5–23.08)	<0.001
LMR (%)	2.01 (0.16–50.76)	2.35 (0.60–8.37)	0.496
NMR (%)	10.96 (2.16–120.25)	8.01 (2.64–26.37)	<0.001

Table 2 Bivariate Analysis of Differential White Blood Cell Counts to Length of Stay

Parameter	Unit	Length of Stay		*p-value*
		Less than 10 days Median (min-max)	10 days or more Median (min-max)	
Lymphocyte	10^3/µL	16.20 (1.3-58.4)	13.00 (1.9-86.3)	0.001
Monocyte	10^3/µL	8.00 (1.3-22.1)	6.80 (0.8-19.7)	0.001
Neutrophil	10^3/µL	74.35 (29.0-95.7)	79.5 (10.7-96.2)	<0.001
Neutrophil-Lymphocyte Ratio (NLR)	%	4.50 (0.5-73.5)	6.20 (0.1-50.4)	<0.001
Lymphocyte-Monocyte Ratio (LMR)	%	2.13 (0.16–8.21)	2.02 (0.25–50.76)	0.659
Neutrophil-Monocyte Ratio (NMR)	%	9.31 (2.39–72.31)	11.23 (2.16–120.25)	0.014

to the protocols established in two previous cohort studies: the REMISI Study and the MEGACRON Study. Both studies received ethical clearance from the Research Ethics Committee of Persahabatan Hospital (64/KEPK-RSUPP/06/2021 for REMISI and 03/KEPK-RSUPP/01/2022 for MEGACRON). All participants in these cohort studies provided informed consent for their involvement in the research.

Result

This study included hospitalized individuals from standard inpatient and intensive care units infected with COVID-19 during the surge in cases caused by the Delta and Omicron variants. The analysis involved data from 463 patients who met the study criteria. The majority of the participants were male, comprising 62.2% of the total. The median age of the participants was 54 years, with a range from 14 to 93 years. Subject characteristics are presented in Table 1.

The LOS was categorized with a cut-off of 10 days. Bivariate tests revealed a significant association between lymphocytes, neutrophils, monocytes, NLR, and NMR and LOS (Table 2).

Logistic regression was performed with adjustment to age, gender, BMI, and comorbidity. The analysis revealed a significant association between monocyte count and length of stay (adjusted OR 0.899; 95% CI 0.840–0.943; p<0.001).

ROC analysis was further performed to evaluate the role of monocyte count as a marker for LOS. The area under the curve (AUC) was found to be 0.595, which suggested that monocyte count is a very poor marker of LOS. A cut-off monocyte count of 8.35 (10^3/µL) demonstrated a sensitivity of 44.8% and a specificity of 69.4% in predicting a patient LOS of less than 10 days. This suggests that lower monocyte counts are associated with longer LOS (Fig. 2).

Table 3 Logistic Regression of Differential White Blood Cell Counts to Length of Stay Adjusted With Age, gender, BMI, and Comorbidity

Variables	OR	95% CI	p-value
Neutrophil	1.009	0.988–1.030	0.406
Lymphocyte	1.032	0.930–1.145	0.555
Monocyte	0.8909	0.840–0.943	<0.001*
NLR	0.980	0.951–1.011	0.204
NMR	0.992	0.968–1.017	0.552

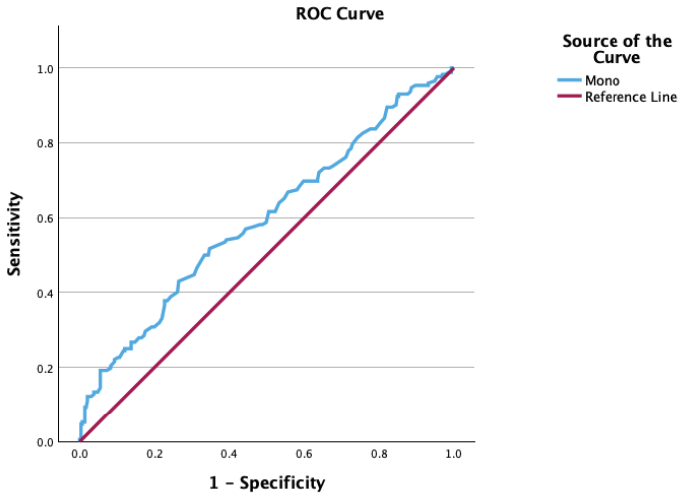

Fig. 2 ROC Curve of Monocyte Count As A Marker of Shorter LOS

Discussion

In this study, a post hoc evaluation was performed on 463 patients with SARS-CoV-2 infection at a national COVID-19 referral hospital. All patients included in this study were confirmed to have SARS-CoV-2 based on a positive RT-PCR assay. The guidelines for hematological laboratory testing in hospitalized COVID-19 cases in Indonesia are crucial tools for disease management and prognosis assessment.[17,18] This study demonstrated a notable relationship between differential white blood cell counts and the LOS in COVID-19 patients. Analysis revealed that lymphocyte, neutrophil, and monocyte counts, as well as the neutrophil-lymphocyte ratio, were significantly associated with a prolonged LOS exceeding 10 days. In particular, monocyte counts higher than 8.35 × 10^3/µL were identified as a predictor of hospital stays of less than 10 days.

Several studies have indicated a correlation between lymphopenia and prolonged hospitalization among COVID-19 patients. A meta-analysis by Tan *et al*. found that lymphopenia was associated with disease severity and could serve as a predictor of prolonged hospital stays.[19] Additionally, a retrospective cohort study by Zhou *et al*. demonstrated that lymphopenia was a common feature among hospitalized COVID-19 patients, with lower lymphocyte counts observed in those with severe illness and longer hospital stays.[20] Similarly, this bivariate tests revealed a significant association between lymphocyte levels and hospital stays longer than 10 days, with patients exhibiting lower median lymphocyte counts. This association between lymphopenia and prolonged hospitalization underscores the importance of monitoring lymphocyte levels as a prognostic indicator for disease severity and patient outcomes in COVID-19 cases.

On the other hand, the relationship between monocyte levels and length of hospital stay in COVID-19 patients has been less extensively studied. Emerging evidence suggests that monocyte alterations may also affect disease progression and outcomes. Several studies, including those by Zhao *et al*. and Liu *et al*., have shown that monocytopenia is associated with a shorter length of hospital stay. Patients with lower monocyte counts upon admission experience milder disease and shorter hospitalizations compared to those with normal monocyte levels,[21,22] which were different to the findings in this study. However, another study conducted by Kilercik *et al*. reported a complex relationship between monocyte count and COVID-19. In severe cases, there may be a depletion of monocyte counts, possibly due to selective recruitment of monocytes during the development of acute respiratory distress syndrome (ARDS) and the presence of microbial superinfections. This leads to a "shift-to-the-left" process in white blood cell production and alterations in myeloid cell homeostasis.[23,24] Although it should be highlighted that the AUC in this

study was poor, the use of monocytes as a marker should be interpreted cautiously, and other markers (i.e., differential white blood cell counts and inflammatory markers) may be beneficial as adjuncts.

Elevated neutrophil counts and an increased NLR have been identified as potential indicators of prolonged hospitalization among COVID-19 patients.[25] Neutrophilia, characterized by elevated neutrophil levels, is associated with severe COVID-19 disease and adverse outcomes. Studies, such as the one conducted by Liu *et al*., have reported that higher neutrophil counts are significantly correlated with disease severity and prolonged hospital stays in COVID-19 patients.[20] Additionally, research by Lagunas-Rangel emphasized the prognostic value of NLR, indicating that elevated NLR levels can predict disease severity and unfavorable clinical outcomes in COVID-19 cases.[26] The NLR reflects the balance between pro-inflammatory neutrophils and lymphocytes involved in the immune response, and its elevation may signify dysregulation of the immune system and heightened inflammation, leading to a more severe disease course and extended hospitalization periods. This study similarly revealed a significant association between neutrophil levels and NLR with LOS, showing that patients with an LOS greater than 10 days had higher median neutrophil and NLR values.

This study has several limitations. First, as an observational study conducted at a single research center, the findings may not be readily generalizable to other settings, particularly where patient characteristics differ significantly, thereby impacting external validity. Additionally, there is a potential for selection bias, as the sample was chosen based on the completeness of available data, which may have been more pronounced during the Omicron wave of COVID-19. The analysis was performed comprehensively without any subset analysis to mitigate this concern and preserve data validity,

In summary, this study found that monocyte counts may serve as a potential marker for the length of stay in COVID-19 patients. These findings could provide valuable insights for optimizing patient care and resource allocation during the pandemic.

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Original Article

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Tacrolimus Therapy Among Steroid-Resistant Nephrotic Syndrome Children: A Preliminary Study in West Java, Indonesia

Ahmedz Widiasta,[1,3] Kurnia Wahyudi,[2,4] Dedi Rachmadi[1,3]

[1]Department of Child Health, Faculty of Medicine, Universitas Padjadjaran-Dr. Hasan Sadikin General Hospital Bandung, Indonesia
[2]Department of Public Health, Faculty of Medicine, Universitas Padjadjaran, Indonesia
[3]Genetic Research Center, Faculty of Medicine, Universitas Padjadjaran, Indonesia
[4]Community Health System Research Center, Faculty of Medicine, Universitas Padjadjaran, Indonesia

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Correspondence:
Ahmedz Widiasta,
Department of Child Health, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital Bandung, Indonesia
Email: ahmedzwidiasta@gmail.com

Abstract

Objective: To explore the outcomes of Tac therapy for Steroid-Resistant Nephrotic Syndrome (SRNS) and its implication in reducing the number of CKD events.

Methods: An open, prospective, cohort study was conducted at a tertiary hospital in Bandung, West Java, Indonesia. Children (age 1–18 years old) with steroid and cyclophosphamide resistant nephrotic syndrome were enrolled in this study. Blood pressure, urinary protein, serum ureum, and creatinine levels were measured every month, Tac and soluble urokinase plasminogen activator receptor (supaR) levels were assessed at the 0, third, and sixth months.

Results: Ten of fifteen subjects enrolled in this study got better within 3–6 months with a trend of decreasing suPAR level and proteinuria, as well as stable blood pressure and serum creatinine and ureum level. During treatment, no side effects of the subjects were found with the Tac level maintain safely.

Conclusion: Tac is an effective and safe agent in treating SRNS, especially for those do not respond well to an alkylating agent.

Keywords: Developing-country, soluble urokinase plasminogen activator receptor, steroid-resistant nephrotic syndrome, tacrolimus

Introduction

Steroid-resistant nephrotic syndrome (SRNS) is a significant issue in pediatric nephrology. It adversely affects children's quality of life, leading to stunted growth, chronic kidney disease (CKD), and potentially end-stage renal disease. In Indonesia, treatment primarily involves cyclophosphamide (CPA), an alkylating agent, or cyclosporine A (CyA), a calcineurin inhibitor, as these are the only drugs well-covered by Indonesian national insurance. The Kidney Disease Initiative Global Outcome (KDIGO) no longer recommends CPA for resistant steroid nephrotic syndrome.[1]
Calcineurin inhibitors (CNIs) are first-line agents recommended by KDIGO for treating SRNS. However, studies on the safety and efficacy of these agents in developing countries remain limited.[1] There is insufficient data from Indonesia regarding the treatment of steroid-resistant nephrotic syndrome with newer drugs like tacrolimus (Tac) or rituximab. As a CNI, Tac is known to be effective and has fewer side effects compared to other immunosuppressive agents. Both Tac and CyA have strong evidence supporting their use in treating SRNS, but some studies revealed that tacrolimus has fewer side effects.[2] Treatment with CyA presents challenges in developing countries, particularly in monitoring blood CyA levels, which should be routinely checked to avoid interstitial nephritis.

Compared to CyA, Tac is more potent in

cytokine suppression and appears to cause less renal toxicity.[1,2] Calcineurin inhibitors (CNIs) are commonly used as first-line agents in children with SRNS. Tac (FK506) acts by inhibiting calcineurin through its association with FK506-binding protein (FKBP). The initial rationale for using CNIs in SRNS stemmed from their immunomodulatory properties, and recent experimental evidence shows that calcineurin is expressed by podocytes. Furthermore, CNIs, or podocyte-specific inactivation of calcineurin, stabilizes the podocyte actin cytoskeleton and reduces proteinuria in response to glomerular injury.[3,4] Unfortunately, prolonged use of Tac has been associated with interstitial fibrosis. In a retrospective single-center study, Tac induced complete remission in 15 of 16 patients (94%) after a median of 120 days of therapy.[5,6]

Therapy with Tac, accompanied by a tapering dose of prednisolone, appears to yield quicker remission than treatment with CYC along with prednisone.[7]prospective cohort study enrolled Chinese adults with SD-MCNS. At the start of the study, we administered TAC or intravenous CYC together with prednisone (0.5 mg/kg/day However, the availability of this drug in some developing countries is limited for treating SRNS.

The best assessment of therapeutic efficacy involves conducting periodic laboratory tests of blood, urine, and kidney biopsy. Most parents of patients in Indonesia are reluctant to subject their children to repeated kidney biopsies, creating a need for alternative examinations that can effectively monitor kidney histology. Recent studies have highlighted the usefulness of soluble urokinase plasminogen activator receptor (suPAR) biomarker in estimating and distinguishing SRNS clinically.[8–12] SuPAR is the circulating form of a glycosylphosphatidylinositol-anchored three-domain membrane protein that is expressed on various cells, including immunologically active cells, endothelial cells, and podocytes.[13]and calcineurin inhibitors (CNIs

The authors aimed to determine the outcomes of tacrolimus administration as therapy for SRNS that did not respond to alkylating agents. To the authors' knowledge, there is still very limited research on this subject, with no such studies conducted in Indonesia.

Methods

This study was conducted as an open, prospective cohort study at a single medical center, Dr. Hasan Sadikin General Hospital, Bandung, Indonesia. The scientific and ethics committee of Hasan Sadikin General Hospital approved the study protocol. The potential risks associated with Tac were communicated to each subject, regardless of their enrollment status in the study.

The researchers enrolled children aged 1 to 18 years with steroid- and cyclophosphamide-resistant nephrotic syndrome, based on the presence of nephrotic-range proteinuria, hypoalbuminemia, and swelling. All patients were admitted to the nephrology department from January 2018 to December 2019. Steroid resistance was defined as a positive urinary protein for more than four weeks of treatment with prednisone or methylprednisolone. Cyclophosphamide (CPA) resistance was defined as persistent urinary protein after six intravenous pulses of CPA treatment. Exclusion criteria included systemic diseases and severe infections prior to Tac therapy. Patients were assigned to the Tac group (oral Tac in combination with oral prednisolone). Oral Tac treatment was initiated at a dose of 0.05 mg/kg/day, divided into two doses over 12-hour intervals, and subsequently adjusted according to the trough blood level, with a target of 4–8 ng/mL. A dosage of 32 mg/m^2 of methylprednisolone was also administeredto the subjects.

The primary outcome measures were complete remission (CR) and partial remission (PR). The secondary outcome measures included suPAR levels, renal function during treatment and follow-up, side effects, and Tac serum levels. Complete remission (CR) was defined as the loss of swelling and a return of proteinuria to the normal range (<0.3 g/day). Partial remission (PR) was defined as a loss of swelling with persistent non-nephrotic proteinuria (0.3–3.5 g/day). The time required for PR was calculated as the duration from the initiation of Tac treatment to the first day PR was observed.

The researchers performed follow-ups monthly for six months. At each visit, complete blood counts, serum levels of creatinine, albumin, alanine aminotransferase (ALT), and urinalysis were obtained, and Tac levels were measured every three months.

Results

Of the 15 enrolled subjects, ten were included in this study. All subjects underwent biopsy by a nephrologist, revealing eight (8) with

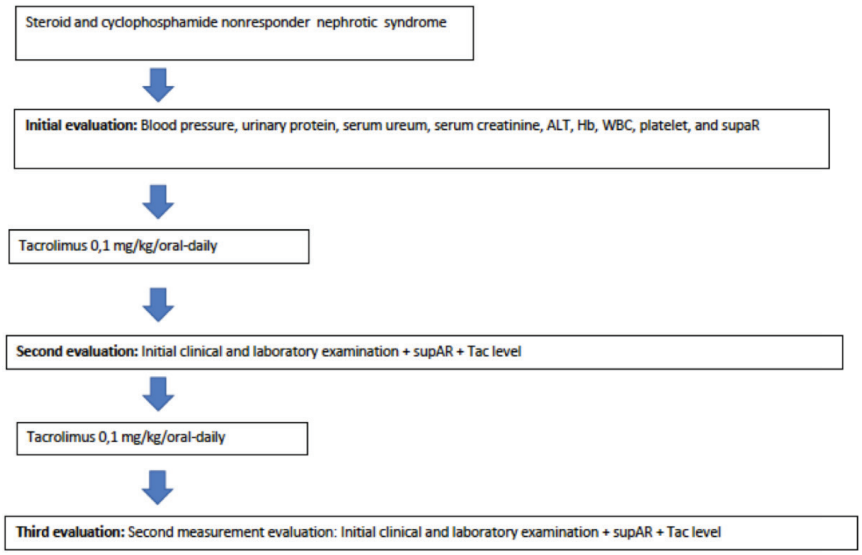

Fig. 1 The Study Flowchart

focal-segmental glomerulosclerosis (FSGS) and two subjects with membranoproliferative glomerulonephritis (MPGN). None of the 15 subjects responded to either steroids or the alkylating agent. Five subjects experienced severe infections during alkylating agent therapy prior to Tac therapy and were subsequently excluded. All ten subjects had suPAR serum levels measured at the initiation of Tac treatment and then again three and six months after treatment commenced. Blood pressure, swelling, proteinuria, complete blood counts, and serum creatinine levels were monitored monthly for six months (Table 1). High suPAR levels were observed at the initiation of Tac treatment, with a wide range of upper and lower limits. By the third month of Tac therapy, the levels decreased, and by the sixth month, they further declined with a narrower range (Fig. 2)

Tac levels at the 0, 3rd, and 6th months of therapy revealed a trend of increasing values,

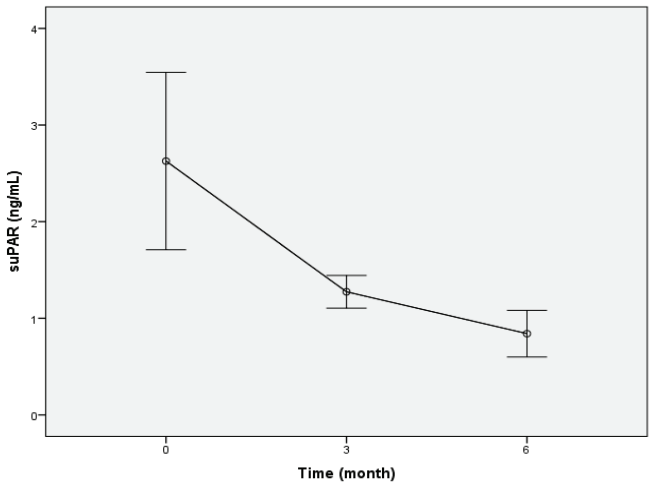

Fig. 2 SuPAR Level During 0, 3, and 6 Months of Tacrolimus Treatment

Table 1 Characteristic, Urinary Protein, Blood Pressure, and suPAR level

Subject Number	Characteristic		Initial			Second visit			Third visit		
	Age (years)	Gender (M/F)	Blood pressure	Urinary protein (g/day)	suPAR (ng/mL)	Blood pressure (3rd month)	Urinary protein (g/day)	suPAR	Blood pressure (6th month)	Urinary protein (g/day)	suPAR (ng/mL)
SRNS-Tac-1	9	M	Hypertension stage 2	3.6	5.18	Hypertension stage 2	3.0	1.67	Hypertension stage 2	2.5	1.66
SRNS-Tac-2	16	F	Normotensive	3.8	1.97	normotensive	2.0	1.32	normotensive	0.4	0.93
SRNS-Tac-3	8	M	Hypertension stage 2	3.6	2.44	normotensive	0.3	1.09	normotensive	0.1	1.02
SRNS-Tac-4	9	M	Hypertension stage 1	3.7	2.02	Hypertension stage 1	0.7	1.66	Hypertension stage 1	0.3	0.82
SRNS-Tac-5	9	M	Hypertension stage 2	3.8	1.94	normotensive	0.5	1.16	normotensive	0.2	0.53
SRNS-Tac-6	11	M	Hypertension stage 2	3.9	2.33	Hypertension stage 2	2.0	1.07	Hypertension stage 1	0.7	0.72
SRNS-Tac-7	12	M	Hypertension stage 2	3.7	1.89	normotensive	0.9	1.35	normotensive	0.6	0.79
SRNS-Tac-8	10	M	Hypertension stage 2	3.6	1.74	Normotensive	0.3	1.31	normotensive	0.1	0.41
SRNS-Tac-9	9	M	Hypertension stage 2	3.6	1.89	Normotensive	0.8	1.02	Hypertension stage 1	0.5	0.75
SRNS-Tac-10	2	M	Hypertension stage 1	3.7	4.87	hypertension stage 1	0.9	1.09	hypertensive stage 1	0.4	0.78
Mean				3.7	2.63		1.14	1.27		0.84	
Median				3.7	1.99		0.8	1.24		0.79	

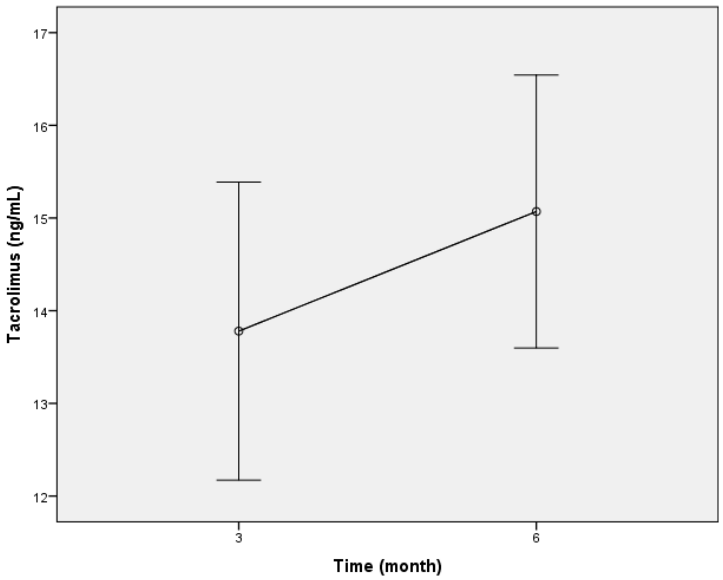

Fig. 3 Tac Level During Third and Sixth Months of Tac Treatment

remaining within the therapeutic range (Fig. 3)

During treatment, no side effects, including decreases in hemoglobin, white blood cell, or platelet counts, or increases in liver enzymes, were observed (Table 2). Monthly monitoring of serum Tac levels showed that no subjects experienced levels lower than 10 ng/mL or greater than 20 ng/mL (Fig. 2).

Discussion

This study reflects that Tac, as a CNI, is an effective agent in treating SRNS, which aligns with the KDIGO recommendation.[1] Some developing countries, such as Indonesia, have low economic resources, making it challenging for many individuals or health insurance plans to cover the costs of CNIs. In these countries, using CPA as an alkylating agent may offer a better solution that balances medical and economic aspects. Although the remission rate of an alkylating agent is lower than that of CNIs in treating SRNS, Indonesia has two CNIs—Tac and CyA—readily available. Previous studies indicate that both Tac and CyA positively impact SRNS, but Tac has a better cosmetic effect.[5] Falkiewicz *et al*. reported that Tac-treated patients recovered significantly faster from tubular phosphate reabsorption impairment compared with CyA-treated recipients. Tac-based immunosuppression also led to better kidney allograft function during a 2-year observation period.[14]

There were 15 subjects in the study; however, five were excluded due to severe infections while undergoing CPA therapy prior to Tac treatment. Four of these subjects experienced severe malnutrition and bronchopneumonia, and one patient died from severe bronchopneumonia. The remaining ten subjects had good nutritional status, excellent medication compliance, and no severe infections during Tac therapy. All patients showed improvement within 3–6 months without any side effects. Their CKD did not worsen, likely related to Tac's immunosuppressive effect on cytokine release.[15] . It may also stabilize the actin skeleton in podocytes and improve foot process motility, preventing podocyte-loss-related proteinuria by inhibiting the dephosphorylation of synaptopodin, which is an initial step for cathepsin-L-mediated degradation of synaptopodin.[16]

Tac is a macrolide antibiotic with immunosuppressive properties. Its mode of action is similar to that of CyA, although the two are structurally unrelated. Tac primarily affects gene expression in target cells by binding to an immunophilin, FK506 binding protein (FKBP). This complex inhibits calcineurin phosphatase, impeding calcium-dependent events such as interleukin-2 (IL-2) gene transcription, nitric oxide synthase (NOS) activation, cell degranulation, and

Table 2 Laboratory Profile During Tac Therapy

Subject Number	Initial						Third month					Sixth month				
	Hb (g/dL)	WBC (x10 mm^3)	Platelete (x10^3 mm^3)	Serum creatinine (mg/dL)	Serum ureum (mg/dL)	ALT (U/L)	Hb (g/dL)	WBC (x10 mm^3)	Serum creatinine (mg/dL)	Serum ureum (mg/dL)	ALT (U/L)	Hb (g/dL)	WBC (x10 mm^3)	Serum creatinine (mg/dL)	Serum ureum (mg/dL)	ALT (U/L)
SRNS-Tac-1	11.5	8.3	320	0.6	35	21	11.4	8.3	0.7	37	25	11.4	8.2	0.7	38	27
SRNS-Tac-2	11.2	9.1	220	0.9	39	31	11.3	9.0	0.8	41	32	11.2	9.0	0.9	40	34
SRNS-Tac-3	10.4	9.9	490	0.8	28	23	10.2	9.8	0.9	27	24	10.3	9.7	0.8	33	27
SRNS-Tac-4	13.2	9.1	470	0.9	39	34	13.0	9.1	0.8	38	37	12.9	9.2	0.8	40	38
SRNS-Tac-5	10.7	8.9	390	0.8	35	13	10.4	8.8	0.8	39	16	10.5	8.8	0.8	41	19
SRNS-Tac-6	11.7	8.8	450	0.9	40	15	11.5	8.7	0.7	38	18	11.3	8.6	0.8	39	20
SRNS-Tac-7	12.1	7.9	350	0.8	43	19	12.1	7.8	0.9	40	21	12.0	8.1	0.8	41	22
SRNS-Tac-8	11.9	9.4	500	0.7	46	24	11.8	9.5	0.8	45	27	12.0	9.4	0.9	45	29
SRNS-Tac-9	11.8	9.6	460	0.9	38	34	11.9	9.5	0.9	39	35	12.0	9.3	0.8	45	37
SRNS-Tac-10	10.7	9.3	370	0.8	35	19	12.0	9.4	0.8	39	21	11.6	9.5	0.8	44	21

apoptosis. Additionally, Tac enhances the actions of glucocorticoids and progesterone by binding to FKBPs within the hormone-receptor complex, preventing degradation. This agent may also enhance the expression of the transforming growth factor-β1 (TGFB1) gene in a manner analogous to CyA, inhibiting T-cell proliferation in response to T-cell receptor ligation. T cell-mediated cytotoxicity is impaired, while B cell growth and antibody production are indirectly affected by the suppression of T cell-derived growth factors; however, antigen presentation appears to be spared.[15,17]

This study revealed that all subjects experienced partial remission (PR) during three months of Tac therapy, after having been on a steroid in combination with CPA for six months without any remission prior to Tac therapy. Six subjects experienced improved blood pressure during the six months of Tac therapy, accompanied by angiotensin-converting enzyme inhibitor administration. All subjects showed a decrease in suPAR levels, indicating that their condition was less likely to progress to end-stage renal disease. SuPAR is a biomarker that may signify CKD progressivity.[13,18,19]

The outcomes observed in this study were consistent with reports from Gulati *et al.*[20] that Tac is superior to CPA in treating SRNS, and from Butani *et al*.[5] that Tac is superior to CPA in treating SRNS, and from Butani *et al*. that Tac is safe and effective in inducing remission in children with SRNS. However, the present study uniquely monitored clinical improvement using suPAR as a novelty. SuPAR was selected as a monitoring tool because its non-invasiveness offers a viable option for subjects who do not have parental permission for repeated kidney biopsies. Furthermore, previous studies have shown that podocyte αvβ3 integrin activity is low under normal conditions but can be enhanced by ligands on podocytes,[21] steroid and cyclophosphamide (CPA, such as suPAR or its soluble form, suPAR.[13] This results in the reorganization of the podocyte actin cytoskeleton and foot process effacement, leading to proteinuria—a hallmark of many primary glomerular diseases.[15]

Tac showed better outcomes because it resulted in earlier complete remission and lower treatment withdrawal rates than CPA. The suggestion is that calcineurin inhibitors (CNIs) are more effective in inducing complete remission in SRNS compared to alkylating agents. The patients in this study did not experience any side effects, which is consistent with other reports indicating that Tac is very safe and has a low risk of nephrotoxicity.[13] Tac level measurements were performed every three months due to the high cost of this examination, which is not well covered by Indonesian national health insurance. The results revealed that Tac levels at the start of therapy, the third month, and the sixth month remained within the therapeutic range. A wide upper and lower limit interval was observed at the initial stage, which narrowed in the third and sixth months, likely due to the variability in FKBP among subjects. The researchers administered a Tac dose of 0.1-0.15 mg/kg BW, along with an oral tapering dose of methylprednisolone. Other laboratory results were normal. This study demonstrated that Tac yields positive results in treating SRNS without any side effects, based on clinical and laboratory monitoring, whereas CPA can cause side effects such as bone marrow suppression, gonadal failure, and malignancy.[21] Patients maintained stable levels of hemoglobin, white blood count, platelet count, and liver enzymes, with some experiencing no cosmetic disturbances. However, some patients did experience cosmetic issues like hirsutism and striae, whichmight be caused by long-term steroid therapy. The most common complications of nephrotic syndrome in children are infections, arising from both the disease itself and immunosuppressive agents. All ten patients did not experience any infections during Tac treatment, whereas five excluded subjects had bronchopneumonia before starting Tac. Another benefit of using Tac for SRNS therapy is that patients have a more straightforward and cost-effective accommodation, as Tac is administered orally and does not require hospitalization. Indonesia has a heterogeneous geographic profile, making accommodation costs a significant barrier to successful treatment. All subjects successfully tapered off steroids and did not experience any relapses during the tapering process, while serum Tac levels were maintained within a therapeutic range, minimizing the risk of interstitial nephritis. In Indonesia, difficulties in achieving repeated kidney biopsies from parents are often encountered; hence, the suPAR measurement could serve as an alternative evaluation tool for assessing whether the SRNS is improving. This study enhances the evidence regarding Tac treatment in SRNS, particularly in developing countries, as previous studies on Tac in SRNS were mostly conducted in developed

countries, which have better drug availability and national insurance coverage.[2]

Tac, in combination with a steroid, is an effective and safe treatment for SRNS, even in cases that are unresponsive to steroid and CPA combinations. The time to achieve complete remission and the side effects of Tac are better than those of CPA; however, more funding and subjects are needed to continue this cohort study with a larger sample size. This report could provide strong evidence to propose Tac as the first-line option covered by Indonesian national insurance for the treatment of children with SRNS, and it may also be a first step toward conducting multicenter randomized controlled trials on the outcomes of SRNS treatment with Tac.

This study provides valuable information, especially for pediatric nephrologists in developing countries, as it demonstrates the success of using Tac in treating SRNS in children with a low risk of clinical toxicity.

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Case Report

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Ifosfamide-Induced Encephalopathy in Relapsed Lymphoma: Report of Two Cases

Davin Takaryanto, Gusti Fungani Harti, Amaylia Oehadian

Department of Internal Medicine, Faculty of Medicine Universitas Padjadjaran-Dr. Hasan Sadikin General Hospital Bandung, Indonesia

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Correspondence:
Davin Takaryanto,
Department of Internal Medicine, Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital Bandung, Indonesia
Email:
takaryantodavin.md@gmail.com

Abstract

Objective: Ifosfamide is a chemotherapeutic drug available for various malignancy, including lymphoma. Ifosfamide has adverse effects including myelosuppression, nephrotoxicity, hemorrhagic cystitis, and neurotoxicity. Encephalopathy is a severe manifestation of neurotoxicity due to ifosfamide, with an incidence of 10–40%. This study aimed to report two cases of ifosfamide-induced encephalopathy.

Case: This case studies reported two relapsed lymphoma patients with almost similar characteristics who received ifosfamide chemotherapy. The first case of 48-year-old woman with relapsed High-Grade B-cell lymphoma stage IIIBE while the second case of 38-year-old woman with relapsed non-hodgkin lymphoma. The first case showed a good outcome with improvement in consciousness 48 hours after stopping ifosfamide and thiamine, while the second case experienced tumor lysis syndrome, leading to the death of the patient.

Conclusion: Mechanism of ifosfamide-induced encephalopathy remains unclear, with the hypothesis from the neurotoxic effects of the resultant metabolite chloracetyldehide. Radiology examination of the brain and electroencephalography is required to rule out other differential diagnoses. Early recognition of adverse effects, followed by immediate discontinuation of ifosfamide, administration of therapy, such as methylene blue and/or thiamine, and supportive treatment usually produced good outcomes.

Keywords: Encephalopathy, ifosfamide, lymphoma, thiamin

Introduction

Ifosfamide is an alkylated oxazaphosphorin derivative with a structural formula that resembles cyclophosphamide. It has been used as chemotherapy for several types of tumors, including ovarian, testicular, cervical, head and neck cancers, lymphoma, and sarcoma.[1–4] Adverse effects of ifosfamide include myelosuppression, nephrotoxicity, hemorrhagic cystitis, and neurotoxicity.[3–5] Neurotoxicity may manifest as extrapyramidal disturbances disorientation, agitation, seizures, confusion, lethargy, and hallucinations, but rarely progresses to coma or causes permanent brain damage or death.[2,6] The incidence of ifosfamide-induced encephalopathy ranges from 10% to 40%.[4,6] These conditions can occur within 2 to 146 hours after intravenous ifosfamide administration and may improve within 48 to 72 hours after the discontinuation of therapy.[1,4,6] Poor performance status, renal impairment, high cumulative dose, history of brain metastases, and hypoalbuminemia are risk factors for neurotoxicity.[2,7,8]

The pathophysiology of the ifosfamide-

induced encephalopathy remains unclear. Several theories suggest that the resultant products of ifosfamide metabolism, such as chloroacetaldehyde and chlorotilamine, act as neurotoxic agents capable of penetrating the blood-brain barrier.[6,9] Based on previous studies, methylene blue, thiamine, and albumin are the treatment options for this condition.[2,6,10] This study aims to report cases of ifosfamide-induced encephalopathy in patients with relapsed lymphoma.

Case(s)

The first patient was a 48-year-old woman with relapsed high-grade B-cell lymphoma stage IIIBE. She had been diagnosed with lymphoma two years before. The patient underwent intra-abdominal tumor resection and colostomy, followed by the R-CHOP chemotherapy regimen (Rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone), achieving a complete response one year earlier. She was subsequently treated with the ICE chemotherapy regimen (ifosfamide, carboplatin, etoposide)The patient had a colostomy, and her vital signs and other physical examinations were within normal limits, with an Eastern Cooperative Oncology Group (ECOG) performance status of 2. Laboratory results indicated mild thrombocytopenia (Table 1), and chest x-rays were normal (Fig. 1).

The patient began chemotherapy with 100 mg/m^2 of intravenous etoposide on the first day. On the second day, the patient received a second dose of 100 mg/m^2 of etoposide, along with 5 AUC of carboplatin and 5,000 mg/m^2 of ifosfamide, administered intravenously. The final dose of 100 mg/m^2 of etoposide was given on the third day. There were no signs of fever or seizures on the fourth day and thereafter. During treatment, indicators of tumor lysis syndrome were monitored, and the results were normal (Table 1). However, on the fourth day, the patient became somnolent and was moved to a semi-intensive care unit for treatment.

There were no focal neurological deficits noted during the physical examination. A head computed tomography (CT) scan with contrast revealed no structural abnormalities (Fig. 2). The patient received 100 mg of thiamine intravenously every 4 hours, without methylene blue (MB) administration due to its unavailability. She became fully conscious after 48 hours, on the seventh day of treatment. Unfortunately, the patient developed neutropenia as a side effect of chemotherapy. She subsequently experienced sepsis from pneumonia and died after two weeks of treatment due to respiratory failure.

The second patient, a 38-year-old woman diagnosed with relapsed non-Hodgkin lymphoma involving the kidneys and lungs, was admitted to the hospital with abdominal pain that had persisted for the last four months. She had previously undergone chemotherapy with the R-CHOP regimen (Rituximab, cyclophosphamide, doxorubicin, vincristine,

Table 1 Laboratory Results (Patient No. 1)

Parameter	Day 1*	Day 4	Day 7
Hemoglobin (g/dL)	11.2	9.6	8.5
Hematocrit (%)	34.2	29.9	26.3
Leukocyte (/uL)	8,830	10,210	12,000
Platelet (/uL)	118,000	131,000	97,000
Absolute neutrophil (/uL)	6,710	9,904	10,680
Ureum (mg/dL)	28.0	39.0	61.0
Creatinine (mg/dL)	1.11	0.88	1.21
Uric acid		4.2	6.0
Potassium (mEq/L)		4.6	3.6
Calcium (mg/dL)		4.95	4.81
Phosphor (mg/dL)		3.64	4.18

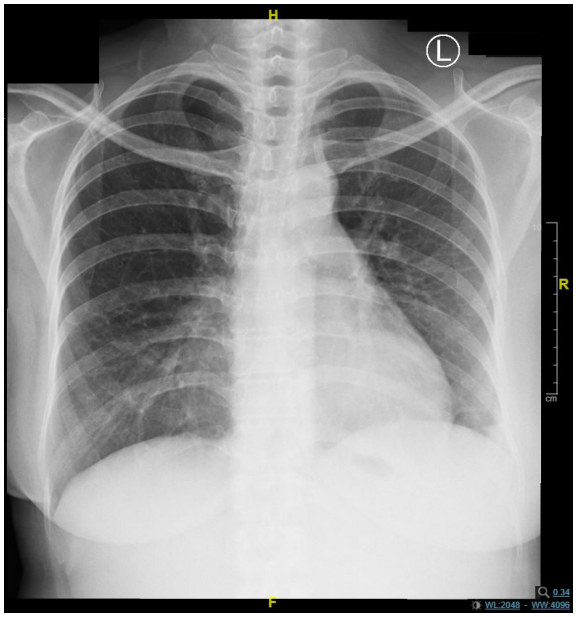

Fig 1. Chest X-ray (Patient No. 1)

and prednisone), achieving a complete response in 2019, and was scheduled to undergo chemotherapy with the ICE regimen. Upon admission, the patient had normal vital signs, but her ECOG performance status was rated at 2. She presented with anemia and a palpable abdominal mass. An abdominal CT scan revealed a mass measuring 20 cm in diameter (Fig. 3). Furthermore, she exhibited anemia and abnormal kidney function (Table 2), and a chest X-ray indicated features of pulmonary involvement (Fig. 4).

The patient underwent chemotherapy at the same dose as patient No. 1. On the third day of chemotherapy, patient No. 2 became agitated and difficult to communicate with, but did not show seizures or overt focal neurological deficits. The patient continued treatment in the intensive care unit, receiving intravenous thiamine at a dose of 100 mg every

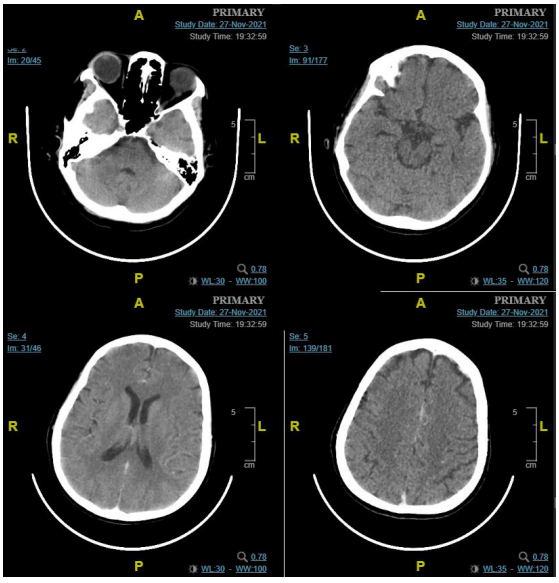

Fig. 2 Head Computed Tomography (CT) with Contrast (Patient No. 1)

Table 2 aboratory Results for Second Patient

Parameter	Day 1*	Day 3	Day 6
Hemoglobin (g/dL)	10.2		7.2
Hematocrit (%)	30.9		22.6
Leukocyte (/uL)	13840		21030
Platelet (/uL)	328000		344000
Absolute neutrophil (/uL)	11210		20609
Ureum (mg/dL)	52.5	67.9	103.6
Creatinine (mg/dL)	2.52	1.69	1.88
Uric acid	3.6	4.8	8.4
Potassium (mEq/L)	3.6	4.7	6.1
Calcium (mg/dL)		4.51	4.25
Phosphor (mg/dL)		4.68	10.72

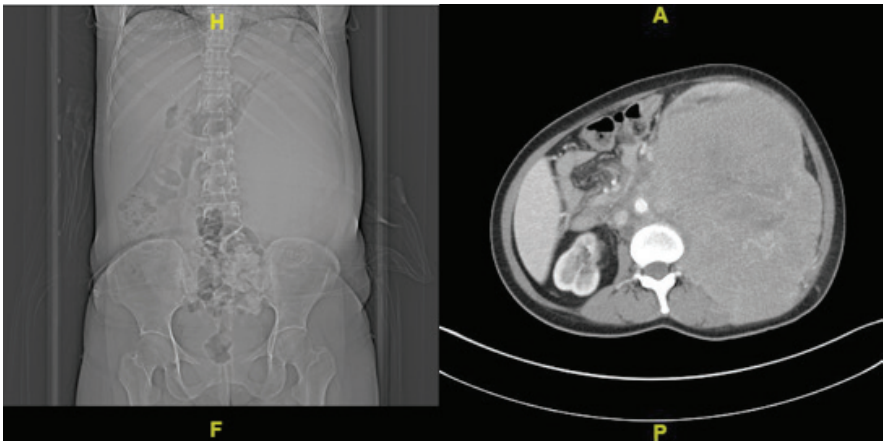

Fig. 3 CT Abdomen with Contrast in Second Patient

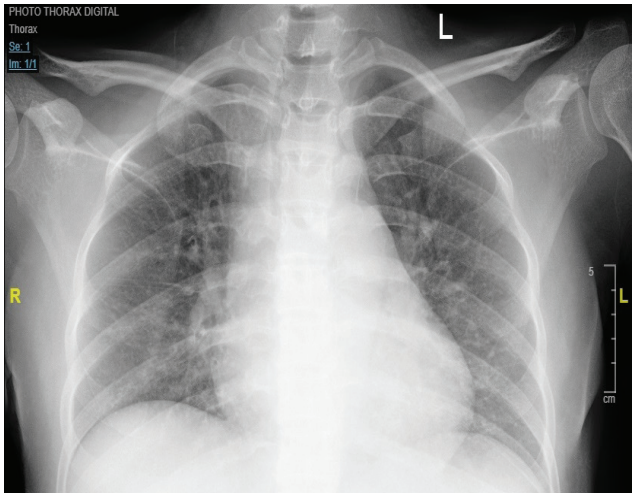

Fig. 4 Chest X-ray (Patient No. 2)

4 hours. Tumor lysis syndrome developed on the second day after chemotherapy. The patient's consciousness was comatose and did not improve; the patient died on the sixth day of hospitalization, following two days of thiamine administration and treatment for tumor lysis syndrome.

Discussion

Ifosfamide metabolism occurs in the liver via cytochrome P450 (CYP450), producing cytotoxic metabolites. The pathophysiological mechanism of encephalopathy remains not fully understood. The neurotoxicity of ifosfamide is attributed to the resultant metabolites of chloroacetyldehyde and chlorotilamine.[6,9,10] Both of these metabolites can penetrate the blood-brain barrier and subsequently inhibit mitochondrial oxidative phosphorylation, reducing glutathione levels in the central nervous system and causing encephalopathy.[3] Tajino *et al*. demonstrated that the ifosfamide dose predisposes patients to a higher incidence of encephalopathy. In a recent study, Yin *et al*. found no significant difference in the incidence of ifosfamide-induced encephalopathy between daily and cumulative doses in patients.[2,7] Both cases in this case study received the same daily dose of ifosfamide and presented with nearly the same symptoms of neurotoxicity. Adverse effects on the central nervous system can occur 2 to 146 hours after ifosfamide administration.[4,6] The first patient showed symptoms of encephalopathy 72 hours after the first dose, while the second experienced encephalopathy 48 hours after the ifosfamide administration. Improvement can occur 48 to 72 hours after stopping therapy.[1,4] The first case showed significant improvement in consciousness 48 hours after discontinuation of therapy, whereas the second case showed no improvement until death. Ifosfamide-induced encephalopathy is a clinical diagnosis with differential diagnoses including infections, other metabolic disorders, and adverse drug interactions. The diagnosis is supported by normal brain radiology to exclude other causes of the patient's altered consciousness. Electroencephalography (EEG) examination reveals typical features of metabolic encephalopathy and correlates with severity. EEG changes can be detected 12–24 hours before symptoms of toxicity appear and improve 24–48 hours before clinical recovery occurs. [1,3,6] A head CT scan with contrast for the first patient was within normal limits; however, the second patient did not undergo brain examination. Decreased consciousness in the absence of obvious focal neurological deficits in a patient receiving ifosfamide is consistent with the clinical diagnosis of ifosfamide-induced encephalopathy.

EEG examinations should be performed routinely in patients with ifosfamide-induced encephalopathy. This condition can present with varying degrees of severity: grade 1 shows vague depressive affect, grade 2 is characterized by prolonged sleep periods, grade 3 includes stuporous consciousness, severe depression, or mild hallucinations, and grade 4 indicates severe hallucinations, seizures, or coma.[1,4,6] Agitation to the point of somnolence became a clear manifestation of grade 3 encephalopathy in both patients. Early evaluation of neurotoxicity after the administration of ifosfamide is needed to prevent the progression of encephalopathy. Several conditions can increase the risk of ifosfamide-induced encephalopathy. Risk factors include female sex, kidney and liver disorders, low albumin levels, a history of platinum consumption, large intra-abdominal tumors, and poor performance status.[1,3,6] Yin *et al*., showed performance status ≥2 and an increase of 1 mg/dL in serum creatinine increase the risk of ifosfamide-induced encephalopathy, whereas an increase of 1 g/dL in albumin reduces this risk.[2]

Both patients had almost the same characteristics, women with a history of intraabdominal tumors and low serum albumin levels (<3.5 g/dL).[1] Patients were managed for ifosfamide-induced encephalopathy by immediately stopping ifosfamide administration, followed by supportive care that included fluid hydration, electrolyte and albumin correction, and the discontinuation of other drugs with potential CNS side effects. Most cases can improve on their own, but healing can be accelerated by the administration of methylene blue (MB) and thiamine. Previous studies have identified MB as therapy and prophylaxis for ifosfamide-induced encephalopathy.[3,4,6] MB acts as a substitute for the electron transport enzyme flavoprotein, restores the oxidative function of nicotinamide adenosine hydrogen dinucleotide (NADH) in the liver, and inhibits the oxidation of monoamine chlorethylamine to chloroacetyldehyde outside the liver.[11] The administration of MB before ifosfamide is crucial as prophylaxis for ifosfamide-induced neurotoxic encephalopathy. Monitoring for MB's adverse effects, such as anaphylactic

shock, Heinz body hemolytic anemia, or serotonin syndrome, is also necessary. Serotonin syndrome caused by MB can lead to disturbances of consciousness that resemble ifosfamide encephalopathy, but it can be differentiated through a thorough examination of the patient's history, physical examination, and the Hunter Serotonin Toxicity Criteria.[4,12–14] The patients in this case study did not receive MB as prophylaxis for ifosfamide-induced encephalopathy because it was not available at Dr. Hasan Sadikin General Hospital in Bandung, Indonesia.

Ifosfamide-induced encephalopathy has features similar to Wernicke's encephalopathy, a syndrome caused by severe thiamine deficiency. Thiamine is considered a therapeutic option and may be used alone or in combination with MB for either treatment or prophylaxis.[15] Chloroacetyldehyde interferes with the function of thiamine pyrophosphorylase (TPP) by preventing TPP from binding to its receptors, without decreasing serum thiamine levels. Thiamine administration is expected to improve the balance of enzyme function in thiamine triphosphate (TPP) and its active form. Thiamine is the treatment of choice for ifosfamide-induced encephalopathy in patients, with thiamine administered at a dose of 100 mg intravenously every 4 hours. Buesa *et al*. explained that thiamine, at doses used in both cases of this study, was the second choice of therapy when MB was unavailable or when there was unresponsiveness after MB administration. [15]

The two cases had different outcomes. The first patient showed an improved response to full consciousness after a single 48-hour thiamine administration, while the second case worsened to death. The clinical worsening of the second case could be due to extensive brain damage due to ifosfamide or tumor lysis syndrome as another side effect of chemotherapy.

Evaluation of the signs and symptoms of acute neurological deficits in patients receiving ifosfamide is particularly important for diagnosing ifosfamide-induced encephalopathic neurotoxicity. Early recognition of the adverse effects of ifosfamide requires immediate management, including discontinuation of the drug and supportive therapy. The clinical diagnosis of ifosfamide-induced encephalopathy is supported by normal brain imaging and EEG showing metabolic encephalopathy, which helps rule out other causes. A routine EEG examination is recommended for diagnosis. Thiamine administration has been shown to provide better outcomes and improve consciousness more quickly. This study has a limitation in that it only discusses two cases of encephalopathy in relapsed lymphoma patients induced by ifosfamide.

In conclusion, the mechanism of ifosfamide-induced encephalopathy remains unclear, with a prevailing hypothesis regarding the neurotoxic effects of the resultant metabolite, chloracetaldehyde. Radiologic examination of the brain and electroencephalography are required to rule out other differential diagnoses. Early recognition of adverse effects, followed by immediate discontinuation of ifosfamide, administration of therapies such as methylene blue and/or thiamine, and supportive treatment usually produce good outcomes.

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