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Erythrocyte Morphology in Sleep-Deprived Students

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ABSTRACT

Late-night wakefulness, defined as staying awake beyond 11:00 PM, may disrupt circadian rhythms and impair homeostatic processes when practiced chronically. College students are particularly susceptible to this behavior due to academic and organizational demands. A descriptive observational study using a quantitative approach, using a purposive sampling technique, based on predetermined inclusion and exclusion criteria. Morphological assessment predominantly showed normocytic normochromic erythrocytes. However, a subset of samples demonstrated normocytic hypochromic features accompanied by mild poikilocytosis (1-2 variations), including echinocyte, elliptocytes, ovalocytes, and dacrocytes. Short-term late-night wakefulness appears to have a more pronounced effect on erythrocyte morphology than on erythrocyte count, with observed alterations remaining mild in severity.

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INTRODUCTION

Staying up late refers to the activity of remaining awake until late at night, particularly after 11:00 PM, a period that should normally be allocated for rest but instead is spent awake (Agamonanza, 2025; Handayani et al., 2026). This habit is commonly associated with activities that require individuals to remain awake throughout the night (Nurlela et al., 2023), such as studying for examinations, completing academic assignments, or participating in organizational activities among university students. When practiced repeatedly, staying up late can disrupt the circadian rhythm and adversely affect the body's homeostatic processes (Fadlilah et al., 2020; Rif'ah et al., 2024).

The habit of staying up late has been shown to influence the circadian rhythm (Bear et al., 2016; Nurlela et al., 2023). The master clock, which regulates bodily functions, enables synchronization between internal biological processes and the external day-night cycle. Disruption of the circadian rhythm may lead to hormonal imbalance, impaired immune function, alterations in blood pressure, sleep disturbances, and changes in erythrocyte and hemoglobin production (Desai et al., 2024). Data published by the National Sleep Foundation (NSF) indicate that the optimal sleep duration for individuals aged 18 to 25 years is 7 to 9 hours daily, with a tolerable range of 6 to 11 hours daily (Hirshkowitz et al., 2015). Staying up late contributes to increased oxidative stress and disturbances in erythropoietin hormone regulation, which subsequently affect erythropoiesis in the bone marrow (Rosdiana & Suryani, 2025). These changes may result in abnormalities in erythrocyte morphology, including anisocytosis and poikilocytosis, as well as alterations in erythrocyte count and hemoglobin concentration in the blood (Azizah & Wibowo, 2025).

High – quality sleep plays an essential role in maintaining immune function, cardiovascular health, hormonal balance, endocrine and metabolic regulation. Among university students, adequate sleep is particularly important for supporting cognitive function, especially memory performance. Sleep deprivation can impair cognitive abilities involved in the formation of long – term memory (Madelu et al., 2025). These findings highlight sleep as a critical factor influencing performance, productivity, and decision-making processes (Walker, 2017).

The effects of sleep deprivation on the hematological system have been extensively investigated using various laboratory parameters. Research conducted by Anggie Kharisma Dewi et al. (2026) demonstrated that staying up late may affect overall biological functions and physiological responses, including the hematological system (Dewi et al., 2026). Furthermore, Agena et al. (2024) reported a study conducted in Kosti, Sudan, involving five men and five women aged 23 – 33 years as the control group. The significantly reduce erythrocyte counts; however, it affected several hematological parameters, including hemoglobin concentration, hematocrit levels, and other blood components. Although staying up late does not always cause significant changes in all hematological, several studies have reported its potential effects on erythrocyte – related parameters (Martini et al., 2018).

Furthermore, reported that sleep disturbances contribute to increased oxidative stress, which may impair blood cell regenerative capacity and hematopoietic stem cell function (Rolls et al., 2016). Quality of life and overall health status are strongly influenced by sleep patterns. Poor sleep quality is associated with an increased risk of health problems, and vice versa (Haryati & Yunaningsi, 2020). Insufficient sleep duration may lead to reductions in hemoglobin and hematocrit levels, thereby affecting erythrocyte production (Lao et al., 2018).

Alterations in erythrocyte morphology represent an important indicator in assessing blood quality and an individual's physiological condition. Anisocytosis and poikilocytosis may reflect disturbances in the erythropoietic process. However, studies specifically examining the relationship between staying up late and changes in erythrocyte morphology and estimated erythrocyte counts remain limited, particularly among student populations. Most previous studies have focused on general morphology through microscopic examination.

In addition, previous findings have shown varying effects of sleep deprivation on erythrocyte parameters. While some studies report no significant reduction in erythrocyte count despite sleep deprivation, alterations in blood cell function and quality were still observed. Therefore, the novelty of the present study lies in its comprehensive analysis integrating erythrocyte morphological features with estimated erythrocyte counts among students with habitual late – night sleeping patterns at the Pekalongan Academy of Medical Laboratory Technology. This approach is expected to provide a deeper understanding of the effects of sleep deprivation on erythrocyte quality and quantity and serve as a basis for future research.

THEORETICAL REVIEW

Sleep, Sleep Stages, and Circadian Rhythm

Sleep is an essential physiological process that supports physical and mental health. During sleep, metabolic activity, heart rate, blood pressure, and body temperature decrease, facilitating recovery and restoration of normal body functions. Conversely, sleep deprivation, commonly caused by staying awake late at night, can disrupt physiological homeostasis and adversely affect multiple body systems (Carskadon & Dement, 2011; Hall & Hall, 2017)

Sleep consists of two major phases: rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep. REM sleep is characterized by increased brain activity, dreaming, and memory processing, whereas NREM sleep comprises three stages (N1, N2, and N3) that progress from light to deep sleep. Deep sleep (N3) is particularly important for tissue repair, immune regulation, energy restoration, and growth hormone secretion (Walker, 2017).

Adequate sleep contributes to cognitive performance, immune function, cardiovascular health, and endocrine balance. Sleep also regulates the circadian rhythm, an endogenous biological clock controlled by the suprachiasmatic nucleus (SCN) of the hypothalamus. Disruption of circadian rhythms due to sleep deprivation or excessive nighttime light exposure has been associated with hormonal imbalance, metabolic dysfunction, impaired immune responses, and alterations in physiological processes (Czeisler & Gooley, 2007; Potter et al., 2016).

Circadian Rhythm and the Impact of Sleep Duration and Quality on Health

The circadian rhythm serves as the body's primary biological clock, regulating physiological processes in synchrony with the light–dark cycle. Its central regulator is the suprachiasmatic nucleus (SCN) located in the hypothalamus, which receives photic signals from the retina. Morning light suppresses melatonin secretion by the pineal gland, promoting wakefulness, whereas darkness stimulates melatonin production and facilitates sleep onset. Repeated sleep deprivation and late-night wakefulness disrupt circadian synchronization, resulting in internal biological desynchronization and impaired cellular restoration during slow-wave sleep (SWS) (Czeisler & Gooley, 2007; Walker, 2017).

Effects of Inadequate Sleep Duration

Both insufficient and excessive sleep duration have been associated with increased mortality risk compared with optimal sleep duration. A large-scale 18-year cohort study involving 322,721 participants from China, Japan, Singapore, and Korea reported that men younger than 65 years who slept less than 5 hours or more than 10 hours per night exhibited the highest mortality risk, with the association weakening with increasing age (Svensson et al., 2021).

Poor sleep duration and quality adversely affect prefrontal cortex function, leading to impairments in attention, memory, and decision-making. Adequate sleep is essential for memory consolidation, facilitating the transfer of information from the hippocampus to long-term cortical storage. Furthermore, sleeping less than 6 hours per night has been associated with a 20% increased risk of myocardial infarction, while sleep duration below 7 hours is linked to a higher risk of heart failure. Chronic sleep deprivation may also contribute to atherosclerosis development and elevate the risk of stroke (Walker, 2017; Yin et al., 2017).

Erythrocyte Morphology and Assesment Methods

Red blood cells (RBCs), or erythrocytes, are biconcave cells specialized for oxygen and carbon dioxide transport (Nurjanah et al., 2025). Their unique morphology provides a high surface area-to-volume ratio, facilitating efficient gas exchange and allowing passage through narrow capillaries (Rosita & Pramana, 2019). The structural integrity and deformability of erythrocytes are maintained by membrane proteins such as spectrin, ankyrin, band 3, and glycophorin (Nigra et al., 2020). Evaluation of erythrocyte morphology is commonly performed using peripheral blood smear (PBS) examination, which remains the gold standard for identifying abnormalities in cell size, shape, and staining characteristics (Ali, 2025; Ardina & Rosalinda, 2018; Goyal et al., 2019). Advances in laboratory technology have also enabled automated morphological assessment using digital hematology analyzers. In addition, erythrocyte count can be determined manually using a hemocytometer or automatically using hematology analyzers, with the latter providing faster and more standardized results (Anjaria & Pursue, 2025; Chen et al., 2025)

Factors Affecting Erythrocyte Morphology

Erythrocyte morphology is influenced by multiple factors, including nutritional status, genetic factors, biological conditions, metabolic status, environmental and lifestyle factors, and pre-analytical variables. Deficiencies in iron, vitamins, or protein may impair hemoglobin synthesis and erythrocyte maturation, resulting in abnormal red blood cell morphology (Mawo et al., 2019; Nurjanah et al., 2025). Genetic mutations affecting erythrocyte membrane proteins or metabolic enzymes can cause erythrocyte dysfunction, including hemoglobinopathies, thereby altering cell shape and stability (Bogdanova et al., 2020; Diederich et al., 2017).

Biologically, erythrocyte aging over approximately 120 days reduces membrane deformability, leading to greater heterogeneity in cell shape, stability, and flexibility (Bogdanova et al., 2020). Under metabolic conditions such as diabetes mellitus, oxidative stress, hemoglobin glycation, and the accumulation of metabolic by-products may damage the erythrocyte membrane and shorten cell lifespan (Hasan & Yunus, 2023; Williams et al., 2023). In addition, smoking, exposure to toxic substances, poor dietary habits, oxidative stress, sleep deprivation, physical inactivity, and residence at high altitude may alter erythrocyte morphology by increasing membrane injury and reactive oxygen species (ROS) production (Brun et al., 2022; Bun et al., 2024; Gligoroska et al., 2019).

Pre-analytical factors are also crucial, as errors in sample collection, mixing, storage, and delayed analysis may cause morphological artifacts. Improperly mixed samples with anticoagulants may undergo hemolysis, crenation, swelling, or cell aggregation, leading to results that do not accurately reflect the patient's true condition (Ashavaid et al., 2008; Iqbal et al., 2023).

Factors Affecting Erythrocyte Count Estimation

Erythrocyte count estimation is influenced by physiological, lifestyle, medical, environmental, and pre-analytical factors. Aging is associated with a decline in bone marrow function and responsiveness to erythropoietin, leading to reduced erythrocyte production. In contrast, infants and young children generally exhibit higher erythrocyte counts due to intrauterine erythropoietin stimulation, followed by a decline at 2–3 months of age and a subsequent increase during adolescence as a result of hormonal changes (Nah et al., 2018). Sex-related differences are also evident, with males typically presenting higher erythrocyte counts than females because of greater muscle mass and testosterone-induced stimulation of erythropoiesis (Gligoroska et al., 2019).

Lifestyle factors significantly affect erythrocyte production. Irregular dietary habits and inadequate nutritional intake may impair erythropoiesis, resulting in reduced hemoglobin concentration and erythrocyte count. (Antosiak-cyrak et al., 2025; Rahmad, 2017). Furthermore, smoking, sleep deprivation, and exposure to unhealthy environmental conditions may adversely affect erythrocyte production and stability (Feriel et al., 2021).

Several medical conditions contribute to alterations in erythrocyte count. Diabetes mellitus promotes oxidative stress and erythrocyte membrane damage, which may reduce red blood cell survival (Hasan & Yunus, 2023; Williams et al., 2023). Likewise, hemoglobinopathies and chronic kidney disease can impair erythropoiesis, accelerate hemolysis, and decrease erythrocyte production (Bogdanova et al., 2020; Gligoroska et al., 2019). Environmental factors, particularly chronic hypoxia associated with high-altitude residence, stimulate erythropoietin production and enhance erythropoiesis, thereby increasing erythrocyte count (Nurjanah et al., 2025).

Pre-analytical variables are an important source of variation in erythrocyte measurements. Errors during blood collection, handling, or sample storage may induce hemolysis, crenation, or cellular damage, leading to inaccurate erythrocyte count estimation (Bogdanova et al., 2020; Feriel et al., 2021; Nurjanah et al., 2025). In addition, patient-related factors prior to sample collection, including fasting status and recent food intake, can influence hematological parameters. Postprandial changes have been associated with alterations in MCH, hematocrit, and red cell distribution width, while significant reductions in hemoglobin and hematocrit have been observed approximately four hours after food consumption (Lippi et al., 2010).

METHODOLOGY

The study employed a descriptive observational research design using a quantitative approach aimed at systematically describing, explaining, and illustrating the observed phenomena. Numerical data obtained from erythrocyte morphology assessments and estimated erythrocyte counts were used to evaluate students with habitual late – night sleeping patterns at the Pekalongan. A descriptive study design was selected to provide an in-depth understanding of erythrocyte morphology and to estimate erythrocyte counts among students who frequently stay up late. Participants were selected using a purposive sampling technique. A total of 30 active students from a population of 185 students at the Pekalongan Academy of Medical Laboratory Technology.

Pekalongan Academy of Medical Laboratory Technology was recruited based on predetermined eligibility criteria. Eligible participants were active students who had completed the survey questionnaire and signed an informed consent form before sample collection. Students who regularly consumed hematinic supplements from the study to minimize potential bias in the research findings.

The study was conducted at the Laboratory of the Pekalongan Academy of Health Analysis from December 2025 to April 2026. The research consisted of three repeated observations of peripheral blood smear samples. Peripheral blood smear specimens were prepared and stained with Wright stain. Microscopic examination was performed by observing ten fields of view at 1000X magnification with immersion oil. Erythrocyte morphology was evaluated for cell size, color, and shape abnormalities, while erythrocyte counts were estimated from the average number of cells observed per high-power field (HPF).

RESULTS

Based on the 30 respondents included in the study, the majority were female, while a smaller proportion were male. According to the survey results regarding the consumption of hematinic supplements, none of the respondents reported taking blood-enhancing medications. Only three respondents reported a history of illness, specifically gastric disorders and asthma, whereas the majority reported no previous medical history.

Sleep duration varied among respondents, ranging from bedtime at 11:00 PM until wake-up time at 02:00 AM, with daily sleep durations generally ranging between 2 – 5 hours/day and 5 – 8 hours/day. Consequently, a considerable proportion of respondents experienced shorter sleep durations than recommended. The frequency of staying up late was most commonly reported as 3 – 4 times/week, indicating that all respondents could be categorized as having a moderate habit of staying up late. The results of the observations are presented in the following table :

Table 1. Estimated Erythrocyte Count Distribution

Range (cells/HPF)	Frequency	Percentage (%)
< 250	5	16,7
250 – 300	20	66,7
> 300	5	16,7

Based on the erythrocyte count estimation performed on 30 respondents, the estimated erythrocyte counts ranged from 200 to 368 cells/HPF. Most respondents were within the range of 250 – 300 cells/HPF, accounting for 20 respondents (66,7%). Meanwhile, five respondents (16,7%) had estimated erythrocyte count below 250 cells/HPF, and another five respondents (16,7%) had erythrocyte counts exceeding 300 cells/HPF.

Table 2. Distribution of Erythrocyte Morphology

Parameter	Category	Frequency	Percentage (%)
Erythrocyte size and color	Normocytic normochromic	14	46,7
	Normocytic hypochromic	16	53,3
Morphological abnormalities	No abnormalities	10	33,3
	Mild poikilocytosis (1-2 variation)	20	66,7
Type of abnormalities	Echinocyte	7	-
	Elliptocyte	6	-
	Ovalocyte	4	-
	Dacrocyte	2	-

Microscopic examination of peripheral blood smears revealed that 16 respondents (53,3%) exhibited normocytic normochromic erythrocytes, whereas 14 respondents (46,7%) showed normocytic hypochromic erythrocytes.

Regarding morphological abnormalities, mild poikilocytosis was identified in 20 respondents (66,7%), while 10 respondents (33,3%) showed no morphological abnormalities. Among the morphological abnormalities observed, the most common cell types were echinocyte, elliptocyte, ovalocyte, and dacrocyte, with echinocytes representing the predominant finding.

DISCUSSION

Microscopic examination of peripheral blood smear preparations demonstrated that the estimated erythrocyte count among students with a habit of staying up late at the Pekalongan Academy of Health Analysts was generally within the normal range, although variations in erythrocyte morphology were observed among individuals. These findings are consistent with the study conducted by Agena et al. (2024), which reported that short-term sleep deprivation did not result in a significant reduction in erythrocyte count (Agena et al., 2024).

Erythrocytes have an average lifespan of approximately 120 days. Therefore, substantial changes in erythrocyte count are unlikely to occur over a short period of sleep deprivation. Consequently, occasional or short-term late-night sleeping habits may not immediately affect erythrocyte quantity. Nevertheless, sleep deprivation can induce physiological stress responses that may influence erythrocyte quality and morphology (Bogdanova et al., 2020; Lew & Tiffert, 2017).

Most respondents exhibited normocytic normochromic erythrocytes, indicating normal erythrocyte size and hemoglobin content. These findings suggest that short-term sleep deprivation may not be sufficient to induce marked alterations in erythrocyte indices or hemoglobin synthesis (Firdayanti et al., 2024; Nurjanah et al., 2025). However, a small proportion of respondents demonstrated normocytic hypochromic erythrocytes, which may reflect physiological stress associated with inadequate sleep or potential pre-analytical factors related to blood smear preparation and staining procedures.

Mild poikilocytosis was observed in several respondents, characterized by the presence of echinocytes, elliptocytes, ovalocytes, and dacrocytes, with burr cells and elliptocytes being the predominant morphological abnormalities. These alterations may be associated with oxidative stress-induced membrane damage, which can affect erythrocyte deformability and structural integrity (Orrico et al., 2023). Nevertheless, the morphological abnormalities identified in this study were generally mild and occurred only in a limited number of samples, suggesting that the observed changes were not clinically significant and may represent transient physiological adaptations to short-term sleep deprivation.

Respondents with shorter sleep duration tended to exhibit greater variability in erythrocyte morphology. This finding indicates a potential relationship between reduced sleep duration and increased susceptibility to morphological alterations in erythrocytes. Overall, the results suggest that sleep deprivation has a greater impact on erythrocyte morphology than on erythrocyte quantity. These findings support the hypothesis that short-term sleep deprivation primarily affects erythrocyte quality rather than erythrocyte count. Furthermore, additional factors such as dietary habits, physical activity, stress levels, and overall health status may have contributed to the observed variations.

The present findings are in agreement with those reported by Agena et al. (2024), who concluded that short-term sleep deprivation tends to influence certain hematological parameters without significantly affecting erythrocyte count. Such discrepancies may be attributed to differences in study populations, sleep deprivation duration, sample characteristics, and methodological approaches.

From a physiological perspective, sleep deprivation is known to activate stress responses and increase the production of reactive oxygen species, leading to oxidative stress and systemic inflammation. These processes may adversely affect erythrocyte membrane stability and function, resulting in morphological changes and reduced cellular resilience (Davinelli et al., 2024). The occurrence of mild poikilocytosis in the present study supports the concepts described in Dacie and Lewis Practical Haematology, which state that erythrocyte shape abnormalities may arise from oxidative stress, metabolic disturbances, and transient physiological conditions (Bain, 2021)

CONCLUSIONS AND RECOMMENDATIONS

The findings of this study indicate that the estimated erythrocyte counts of most respondents remained within the normal range, with predominantly normocytic normochromic erythrocyte morphology. However, mild poikilocytosis characterized by the presence of echinocytes, elliptocytes, ovalocytes, and dacrocytes was observed in some respondents. These findings suggest that short-term sleep deprivation may be associated with subtle alterations in erythrocyte morphology rather than significant changes in erythrocyte count. Therefore, inadequate sleep may affect erythrocyte quality, as reflected by morphological variations, while having minimal impact on erythrocyte quantity.

Based on these findings, maintaining healthy sleep habits is recommended to support overall health and hematological function. Future studies should include larger sample sizes and compare the effects of short-term and long-term sleep deprivation on erythrocyte morphology and count. Furthermore, analytical or experimental studies incorporating additional hematological parameters, such as hemoglobin concentration, hematocrit, erythrocyte indices, and oxidative stress biomarkers, are recommended to provide a more comprehensive understanding of the hematological effects of sleep deprivation. Potential confounding factors, including nutritional status, physical activity, stress levels, and underlying health conditions, should also be considered to improve the validity and reliability of future research findings.

FURTHER STUDY

Despite providing valuable insights, this study has several limitations. First, the observation period for sleep deprivation was relatively short (3 – 7 days), limiting the ability to assess its long – term effects on erythrocytes. Second, potential examination artifacts resulting from blood smear preparation and staining procedures may have influenced the interpretation of erythrocyte morphology. Third, other factors that may have influenced the interpretation of erythrocyte characteristics, such as nutritional status, genetic background, physical activity, stress levels, and overall health conditions, were not comprehensively evaluated and may have acted as confounding variables. Finally, the descriptive study design does not allow for direct inference of a causal relationship between sleep deprivation and erythrocyte alterations.

Furthermore, the limitations identified in the present study may serve as valuable references for subsequent researchers in designing studies that address and overcome these limitations. Provide some conclusions and the implementation of the research results.

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