Reference Range of *Stomatocytes* in Jakarta and Surrounding Areas

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Abstract

Stomatocytes are erythrocytes characterized by a slit-like central pallor. Although they may appear in small numbers under physiological conditions, increased proportions are often associated with pathological states. Establishing population-specific reference intervals is therefore essential to avoid misinterpretation of erythrocyte morphology. This study aims to determine the reference interval for Stomatocyte counts in a healthy population from Jakarta and its surrounding areas, and to assess their relationship with routine hematological and biochemical parameters. A descriptive cross-sectional study was conducted among healthy individuals undergoing health check-ups at UKI General Hospital and East Bekasi Hospital from August 2022 to October 2024. Of 65 samples collected, 54 met the inclusion criteria. Stomatocyte counts were evaluated from peripheral blood smears by counting per 1,000 erythrocytes. Reference intervals were established using the 2.5th to 97.5th percentile method. The overall reference interval for Stomatocytes was 0 - 1.85%. Gender-specific intervals were 0 - 2.0% in men and 0 - 1.7% in women. No significant associations were observed between Stomatocyte counts and complete blood count parameters, liver function tests, or kidney function tests. In conclusion, Stomatocytes may be present at low levels in healthy individuals, with a locally derived reference interval of 0 - 1.85%. These findings highlight the importance of applying clearly defined reference intervals and considering clinical context when interpreting erythrocyte morphology to prevent overdiagnosis.

Keywords: Stomatocytes, Reference Values, Erythrocyte Morphology, Peripheral Blood Smear.

INTRODUCTION

Erythrocytes are responsible for transporting oxygen and carbon dioxide throughout the body tissues. Their biconcave structure provides both flexibility for navigating narrow vascular spaces and a large surface area for efficient gas exchange. However, this structural advantage also makes erythrocytes susceptible to morphological changes under pathological or physiological stress. *Stomatocytes* represent one such abnormality, defined by an elliptical or lip-shaped central pallor that arises from disturbances in transmembrane ion balance or genetic mutations, particularly in the PIEZO1 and KCNN4 genes. (Achfidawati, Elfiah, and Sakinah 2019; Alareeqi et al. 2021; Bissinger et al. 2019; Chen et al. 2023; Hamid, Pakhri, and Adam 2021; Imannual and Harun 2019). Careful evaluation of erythrocyte morphology is therefore essential, as the presence of *Stomatocytes* may reflect underlying membrane instability or systemic disorders, with important implications for *hematological* assessment.

The morphological variation in red blood cells, including the presence of *Stomatocytes*, highlights the need for standardized terminology, recognition criteria, and reporting methods to ensure consistency among laboratories. The International Council for Standardization in Hematology (ICSH) has issued guidelines on the nomenclature and grading of peripheral blood cell morphology, along with updated recommendations for quantifying certain abnormal forms, such as schistocytes, to minimize diagnostic errors (Melzak et al. 2018; Palmer et al. 2015; Zini et al. 2021). Although the ICSH primarily emphasizes schistocyte thresholds rather than *Stomatocyte* counts, its broader framework for standardizing blood smear morphology provides valuable guidance for local reporting practices and for validating findings of rare or low-frequency abnormal cell (Andolfo, Iolascon, and Russo 2025; Palmer et al. 2015; Zini et al. 2021).

Pathobiologically, *Stomatocytes* can reflect impaired erythrocyte membrane permeability and disrupted cation homeostasis (Na^+/K^+) in hereditary conditions such as dehydrated hereditary *stomatocytosis* or hereditary xerocytosis (Andolfo et al. 2025). They may also arise from alterations in membrane lipid composition associated with alcohol-related liver disease, in which *Stomatocyte* formation resolves with improvement in liver function (Ridwan, Agustina, and Makmur 2021). In healthy individuals, cells with *Stomatocyte*-like morphology may occasionally appear as artifacts, particularly due to smear-drying effects. For this reason, evaluation should include multiple smear areas rather than relying on a single field of view (Chen et al. 2023). These considerations reinforce the importance of establishing local reference values for *Stomatocyte* counts, enabling clinicians to distinguish physiological variation from pathological manifestations.

Previous studies report that *Stomatocytes* occur at a prevalence of less than 3% in healthy individuals. In contrast, counts exceeding 5% are commonly associated with pathological conditions such as hereditary *stomatocytosis*, liver cirrhosis, fatty liver, and hemolytic anemia (Körber et al. 2017; Pozdnyakova 2025; Romanenko et al. 2024). At present, Indonesia lacks standardized reference values for *Stomatocytes*, and available data are largely derived from populations in America and Europe. This gap reflects the limited research conducted on *Stomatocyte* morphology within Indonesia. Establishing locally derived reference intervals is therefore essential, given the potential influence of genetic, ethnic, and environmental factors on erythrocyte morphology.

Based on this basis, establishing locally derived reference values is essential to improve diagnostic accuracy in identifying erythrocyte shape abnormalities in Indonesia. Therefore, the

present study aims to determine *Stomatocyte* reference values in a healthy adult population from Jakarta and its surrounding areas, and to examine potential differences according to gender.

METHODS

A descriptive cross-sectional design was employed, with primary data obtained through laboratory examinations and peripheral blood smear analyses. The study was conducted at the Indonesian Christian University General Hospital in Jakarta and a hospital in East Bekasi, with data collection spanning from August 2022 to October 2024. During this period, 65 adult patients undergoing routine medical check-ups were screened for eligibility, of which 54 samples met the inclusion criteria and were included in the final analysis. Eleven samples were excluded due to patient age outside the targeted range of 21 to 60 years or abnormal laboratory results that could confound erythrocyte morphology assessment.

The target population comprised adults undergoing medical check-ups at the two hospitals, representing individuals without acute or chronic conditions that might influence *hematological* parameters. A total sampling technique was applied, including all eligible subjects during the study period, to minimize selection bias and ensure comprehensive data representation. Inclusion criteria required participants to be aged 21 to 60 years with complete and normal laboratory results, including *hemoglobin* (Hb), mean corpuscular volume (MCV), mean corpuscular *hemoglobin* (MCH), mean corpuscular *hemoglobin* concentration (MCHC), red cell distribution width–coefficient of variation (RDW-CV), serum glutamic-pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), urea, and creatinine levels, and no documented history of chronic disease or *hematological* disorders. Exclusion criteria included incomplete medical records, pregnancy or breastfeeding, active smoking or alcohol consumption, ongoing chemotherapy, and abnormal laboratory findings, as these factors may alter erythrocyte morphology and compromise the validity of the results.

The tools and materials used in this study included EDTA tubes, slides, dropper pipettes, staining racks, light microscopes, and staining reagents (methanol, Wright stain, distilled water, immersion oil). Venous blood samples were collected from each subject. For samples meeting the inclusion criteria, peripheral blood smears were prepared using one drop of EDTA blood placed on a slide, followed by fixation and staining with methanol and Wright stain. Smears were examined under a light microscope at 1000x magnification with immersion oil (Chen and Boyle 2017), and images were recorded using a microscope camera. *Stomatocyte* counts were determined by evaluating 1,000 erythrocytes per smear, with the number of

Stomatocytes documented. All results were verified by a clinical pathologist to ensure accuracy (Ridwan et al. 2021).

Data analysis was performed univariately, with reference values calculated using the median and the 2.5th to 97.5th percentile range in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Normality testing and distribution analysis were conducted using SPSS software version 27. For non-normally distributed data, the median was reported as the measure of central tendency, and the percentile range was used to describe the distribution (Liana et al. 2022; Rosida and Hendriyono 2015).

RESULTS

This study included 54 subjects, comprising 37 men (68.52%) and 17 women (31.48%). All participants were confirmed to be healthy based on medical check-ups and laboratory evaluations, which encompassed basic hematology and biochemistry parameters, and met the predefined inclusion and exclusion criteria. The age distribution of subjects ranged from 21 to 60 years.

Table 1 Sample Data Distribution (n=54)

Parameter	Subject	Mean	Median
Age			
21–30 years	11 (20,75%)	23,55	24
31–40 years	11 (20,75%)	36,27	36
41–50 years	22 (41,51%)	45,45	45
51–60 years	9 (16,98%)	54,56	54
Hemoglobin **(Hb)**			
Male	37	14,92 g/dL	14,1
Female	17	13,00 g/dL	12,8
Mean Corpuscular Volume (MCV)	54	84,49 fL	85,25
Mean Corpuscular *Hemoglobin* (MCH)	54	29,79 pg	29
Mean Corpuscular *Hemoglobin* Concentration (MCHC)	54	33,55 g/dL	33,35
Red Cell Distribution Width–Standard Deviation (RDW-SD)	54	39,65 fL	39,9
Red Cell Distribution Width–Coefficient of Variation (RDW-CV)	54	12,78 %	12,7
Urea	54	22,95 mg/dL	20,85
Creatinine	54	0,873 mg/dL	0,87
Serum Glutamic-Pyruvic Transaminase (SGPT)	54	20,92 U/L	19
Serum Glutamic-Oxaloacetic Transaminase (SGOT)	54	21,31 U/L	21

Tabel 2 Median *Stomatocyte*

Stomatocytes	**Number of Patients**	**Median *Stomatocytes* per 1000 Erythrocytes**
Overall	54	3
Male	37	4
Female	17	3

Table 3 Percentage of *Stomatocyte* Findings by Gender

Variable	Percentage (%)
Total	0-1.85
Male	0-2
Female	0-1.7

In this study, *Stomatocytes* were either absent or observed in very low proportions across all peripheral blood smears. The overall percentage range of 0–1.85% indicates that *Stomatocytes* were undetectable in most samples, and when present, they appeared only sporadically, remaining within the upper threshold typically considered normal morphological variation. Stratification by gender revealed a slightly higher maximum percentage in men (0–2.00%) compared to women (0–1.70%); however, this difference was minimal and not clinically significant. The absence of prominent *stomatocytosis* in the majority of samples is consistent with the strict inclusion criteria applied, which ensured that participants had normal *hematological* and biochemical parameters and no history of conditions known to affect erythrocyte membrane stability. These findings suggest that *Stomatocytes* observed in this population represent normal morphological variants rather than pathological abnormalities.

DISCUSSION

The presence of *Stomatocytes* at low percentages in healthy individuals suggests that this erythrocyte morphology represents a physiological variation rather than an inherent pathological condition. *Stomatocytes* are defined by a slit-like central pallor resulting from alterations in the erythrocyte membrane curvature, which may occur transiently due to changes in membrane lipid composition, intracellular ion balance, or environmental factors during sample handling (Perrin, Ranta, and Lesesve 2015; Pozdnyakova 2025). The reference ranges identified in this study, 0-1.85% overall, 0-2% in men, and 0-1.7% in women, are consistent with previous reports describing *Stomatocytes* in up to 3% of erythrocytes in healthy populations (Perrin et al. 2015; Pozdnyakova 2025). The minor gender-related differences observed may reflect gender hormonal influences on erythrocyte membrane fluidity, particularly the effects of *estrogen* and androgen levels on lipid metabolism and ion transport mechanisms such as sodium–potassium ATPase activity. These factors can subtly affect erythrocyte shape without producing clinically significant *hematological* abnormalities.

The low prevalence of *Stomatocytes* observed in this study does not indicate an increased risk of future *hematological* disorders, as isolated *stomatocytosis* at minimal levels is not associated with *hemolysis, anemia*, or impaired red cell function (Fusi et al. 2024; Geekiyanage et al. 2020). Pathological relevance generally arises only when *Stomatocytes* are present in markedly elevated proportions or are accompanied by clinical symptoms, laboratory

evidence of hemolysis, or underlying conditions such as hereditary *stomatocytosis*, liver disease, or alcohol-related toxicity (Pozdnyakova 2025).

Physiologically, *Stomatocytes* may form as a result of disturbances in osmotic balance and cell membrane structure, including genetic mutations (PIEZO1, KCNN4, RHAG) or exposure to certain drugs. However, no clinical or biochemical evidence of pathological *etiology* was observed in this study population (Achfidawati et al. 2019; Flatt and Bruce 2009). Because the distribution of *Stomatocyte* data was non-normal, the 2.5–97.5% percentile method was applied. The use of the median and percentile range was considered more representative than the mean ± 2SD, which is susceptible to distortion by extreme values.

In clinical practice, pathological *stomatocytosis* primarily encompasses hereditary *stomatocytosis* (HSt), a spectrum of hemolytic anemias characterized by increased cation permeability of the erythrocyte membrane and typically associated with hemolysis, anemia, and abnormal laboratory findings (Andolfo et al. 2025; Flatt and Bruce 2009). Beyond hereditary forms, "acquired" *Stomatocytes* may arise in conditions such as liver disease, alcohol use disorder, exposure to certain cationic drugs, and lipid metabolism disorders (Wislöff and Boman 1979; Zini et al. 2021). In this study, the absence of liver or renal function abnormalities and hematologic parameters supports the interpretation that the low frequency of *Stomatocyte* observed does not reflect an active pathological process (Pijpers et al. 2017).

Variation by Gender

The slightly higher median *Stomatocyte* count observed in men (4/1000 erythrocytes) compared with women (3/1000 erythrocytes) may reflect gender-related physiological differences in erythropoiesis and red blood cell turnover, which also influence *hemoglobin* concentration and *hematocrit* values (Bachman et al. 2014; Murphy 2014). Androgen hormones, particularly testosterone, stimulate erythropoietin production and promote erythroid progenitor proliferation, leading to increased red blood cell mass and potentially greater variability in erythrocyte morphology.

In contrast, *estrogen* has been shown to stabilize erythrocyte membrane composition and oxidative balance, which may contribute to reduced morphological variation in women. Additional gender-specific differences in erythrocyte membrane lipid composition, intracellular ion transport, and cell hydration status may also affect the propensity for transient, *nonpathologic stomatocytic* changes.

These subtle morphological variations are generally regarded as physiological and do not indicate underlying *hematological* disease when present at low frequencies. However, given the limited sample size and the predominance of male participants in this study, the

observed difference may reflect sampling variability rather than a true biological effect. This finding should therefore be interpreted with caution and warrants validation in future studies with larger, gender-balanced cohorts and controlled assessment of confounding variables such as age, hydration status, and pre-analytical factors.

Practical Implications

Contextual Laboratory Interpretation.

The identification of very low *Stomatocyte* counts (approximately <2%) in individuals with otherwise normal complete blood count parameters and unremarkable biochemical profiles is most likely attributable to physiological variation or pre-analytical and analytical artifacts rather than to underlying pathology. Factors such as smear preparation technique, drying time, anticoagulant effects, and field selection during microscopic evaluation can influence erythrocyte morphology and lead to the incidental observations of *Stomatocytes*. Accordingly, evaluation across multiple microscopic fields and, when necessary, repeat smear examination are recommended to ensure accurate interpretation before classifying such findings as abnormal (Chen et al. 2023).

Avoidance of Overdiagnosis of Hereditary *Stomatocytosis* (HSt).

The identification of *Stomatocytes* alone, particularly at low frequencies, is insufficient for diagnosing hereditary *stomatocytosis*. Hereditary *stomatocytosis* is a rare *hemolytic* disorder characterized by persistent *stomatocytosis* in conjunction with clinical and laboratory indicators of *hemolysis*, such as *anemia*, reticulocytosis, elevated lactate dehydrogenase, and decreased haptoglobin levels. Comprehensive clinical assessment, detailed family history, and correlation with *hemolytic* markers are essential for accurate diagnosis. When clinical suspicion persists, advanced diagnostic techniques, including osmotic gradient ektacytometry and targeted genetic testing for membrane protein or ion channel mutations, are necessary to confirm the diagnosis (Andolfo et al. 2025; Mottelson et al. 2025).

Consideration of Hepatic and Alcohol-Related Comorbidities.

When *Stomatocytes* are present in significantly elevated proportions, secondary causes should be carefully evaluated. Acquired *stomatocytosis* has been associated with liver disease, chronic alcohol consumption, and exposure to certain medications, all of which can alter erythrocyte membrane lipid composition and disrupt ion transport mechanisms. Consequently, targeted screening that includes liver function tests, medication review, and assessment of alcohol intake is warranted in such cases to distinguish reversible, secondary *stomatocytosis* from inherited red cell membrane disorders (Imannual and Harun 2019; Wislöff and Boman 1979; Zini et al. 2021) .

Limitations

Major limitations include the small sample size and gender imbalance, as well as the failure to evaluate lifestyle factors (smoking, alcohol consumption, physical activity) known to influence erythrocyte morphology. Further studies with larger, balanced samples and strictly standardized pre-analytical protocols (e.g., smear drying time, reading area, anticoagulant) are needed to validate local reference values for *Stomatocyte* frequency in healthy populations and explore its biological determinants (Brihi 2024).

CONCLUSIONS

Low proportions of *Stomatocytes* can be observed as a physiological finding in healthy individuals. This study established a population-specific reference interval for *Stomatocyte* counts in adults from Jakarta and its surrounding areas using the 2.5th–97.5th percentile method, with an overall range of 0–1.85% and gender-specific upper limits of 2.0% in men and 1.7% in women. The lack of significant associations between *Stomatocyte* counts and routine *hematological* parameters, as well as liver and kidney function, indicates that low-level *stomatocytosis* does not suggest underlying pathology. Employing locally derived reference intervals in conjunction with appropriate clinical correlation is crucial for enhancing peripheral blood smear interpretation and minimizing the risk of overdiagnosis in routine laboratory practice.

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