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Reference Range of Stomatocytes in Jakarta and Surrounding Areas

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Abstract

Stomatocytes are a type of abnormal erythrocyte characterized by an elliptical or cleft lip-like central pallor. Physiologically, stomatocytes can occur in minimal numbers; however, an increase in their number is often associated with pathological conditions, such as liver disease, hemolytic anemia, and certain genetic disorders. Determining local reference values for stomatocyte counts in healthy populations is crucial for distinguishing normal variations from pathological conditions, particularly since geographic, genetic, and environmental factors can influence hematological variation between populations. This study aims to determine the reference value of stomatocyte count in a healthy population in Jakarta and its surroundings, and to evaluate its relationship with basic hematological parameters, liver function, and kidney function. This research is a descriptive study with a cross-sectional design. Samples were taken consecutively from healthy individuals undergoing health check-ups at UKI General Hospital and East Bekasi Hospital between August 2022 and October 2024. Of the 65 samples, 54 subjects met the inclusion criteria, namely healthy individuals without a history of hematological, hepatic, or renal disease. Peripheral blood smear examination was performed to calculate the number of stomatocytes per 1000 erythrocytes. Data analysis used SPSS v.27 software with a 2.5–97.5% percentile distribution approach to determine reference values. The reference value for stomatocytes in a healthy population as a whole is 0–1.85%. Based on gender, the reference value for men is 0–2%, while for women it is 0–1.7%. Further analysis showed that the stomatocyte count was not significantly associated with routine hematological parameters, liver function, or kidney function. Stomatocytes can be found in small numbers in healthy individuals, with reference values ranging from 0–1.85% in the population of Jakarta and its surrounding areas. These findings support the need to consider the clinical context and local reference values for interpreting erythrocyte morphology.

Keyword: Stomatocytes, reference values, abnormal erythrocytes, peripheral blood smear.

INTRODUCTION

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Erythrocytes play a role in transporting oxygen and carbon dioxide throughout the body's tissues. They are biconcave in structure. This shape allows them to be flexible when passing through narrow vascular spaces, but it also makes them susceptible to morphological changes. One form of erythrocyte abnormality is stomatocytes, characterized by an elliptical or lip-shaped central pallor area due to transmembrane ion imbalance or genetic mutations, such as those 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).

The morphological variation of red blood cells, including stomatocytes, necessitates standardization of terminology, recognition criteria, and reporting methods to ensure interlaboratory consistency. The International Council for Standardization in Hematology (ICSH) has published guidelines for the nomenclature and grading of morphological features of peripheral blood cells, as well as updated recommendations for the quantitation of some

fragment forms (e.g., schistocytes) to prevent diagnostic misinterpretation (Palmer et al. 2015; Zini et al. 2021). While the ICSH's focus on schistocyte thresholds does not directly establish stomatocyte thresholds, the spirit of standardizing morphology on blood smears provides a framework for local reporting practices and validation of low-number abnormal cell findings (Palmer et al. 2015; Zini et al. 2021).

Pathobiologically, stomatocytes can reflect impaired erythrocyte membrane permeability and cation homeostasis (Na^+/K^+) in hereditary conditions such as dehydrated hereditary stomatocytosis/hereditary xerocytosis (Andolfo, Iolascon, and Russo 2025), as well as changes in membrane lipid composition in alcohol-related liver disease, which can cause stomatocyte shapes and resolve with improvement in liver function (Ridwan, Agustina, and Makmur 2021). However, in healthy individuals, cells with stomatocyte-like morphology can also occasionally appear due to drying artifact, which is why assessment should encompass multiple smear areas and not rely on a single field of view (Anon 2025). This reinforces the urgency of having local reference values for stomatocyte counts so that clinicians can differentiate physiological variations from disease manifestations.

Some references state that healthy individuals have a stomatocyte prevalence of less than 3%. In comparison, an increase in the number exceeding 5% is correlated with pathological conditions such as hereditary stomatocytosis, liver cirrhosis, fatty liver, and hemolytic anemia (Körber et al. 2017; Pozdnyakova 2025; Romanenko et al. 2024). To date, Indonesia lacks a standard reference for stomatocytes, and we still rely on data from populations abroad, such as those in America and Europe. This is because there is limited research on this topic in Indonesia. Determining normal values in Indonesia is very necessary due to the influence of genetic, ethnic, and environmental factors.

Based on this, we need to establish local reference values to improve diagnostic accuracy related to changes in erythrocyte shape in Indonesia. Therefore, this study aims to determine the reference values for stomatocytes in a healthy adult population in Jakarta and its surrounding areas, and to evaluate potential differences in these values based on gender.

METHODS

This study used a descriptive cross-sectional design, and primary data were obtained through laboratory tests and peripheral blood smears. The study was conducted at the Indonesian Christian University General Hospital in Jakarta and a hospital in East Bekasi. Data collection was conducted between August 2022 and October 2024. A total of 65 samples were obtained,

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but only 54 met the inclusion criteria. Samples were excluded due to age and laboratory results that did not meet the inclusion criteria. The target sample was adult patients undergoing medical check-ups at the two hospitals. The sampling technique used a total sampling method, so that all subjects who met the inclusion and exclusion criteria were included in the study sample. The inclusion criteria included patients aged 21–60 years, with complete laboratory test results (Hb, MCV, MCH, MCHC, RDW-CV, SGPT, SGOT, urea, creatinine) within normal limits, and no history of chronic disease or blood cell disorders. Meanwhile, exclusion criteria apply to incomplete medical record data, pregnant or breastfeeding women, a history of active smoking or alcohol consumption, and patients undergoing chemotherapy or with abnormal laboratory results.

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The tools and materials used included EDTA tubes, slides, dropper pipettes, staining racks, light microscopes, and staining equipment (methanol, Wright stain, distilled water, immersion oil). The biological material was a venous blood sample from each subject. If the sample met the inclusion criteria, the study began with the preparation of a peripheral blood smear with one drop of EDTA blood taken from the vein, placed on a slide, and then the blood smear was made and fixed using methanol and Wright stain. The preparation was observed under a light microscope at 1000x magnification using immersion oil. Recording was carried out using a microscope camera. After reaching 1,000 erythrocytes, a search was conducted for stomatocytes. The researcher recorded the number of stomatocytes found. Furthermore, the examination results were verified by one clinical pathologist to ensure accuracy (Ridwan et al. 2021). Data were analyzed univariately by calculating the median and the 2.5th to 97.5th percentile range as reference values, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. Normality tests and data distribution analysis were conducted using SPSS software version 27. If the data were not normally distributed, the median was used as a measure of central tendency and the percentile range as a measure of distribution (Liana et al. 2022; Rosida and Hendriyono 2015).

RESULTS

This study involved 54 subjects, consisting of 37 men (68.52%) and 17 women (31.48%). All subjects were healthy individuals, as determined by medical check-ups and laboratory results, which included basic hematology and biochemistry parameters, and met the established inclusion and exclusion criteria. Subjects were distributed in the age range of 21–60 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			
Male	37	14,92 g/dL	14,1
Female	17	13,00 g/dL	12,8
MCV	54	84,49 fL	85,25
MCH	54	29,79 pg	29
MCHC	54	33,55 g/dL	33,35
RDW-SD	54	39,65 fL	39,9
RDW-CV	54	12,78 %	12,7
Urea	54	22,95 mg/dL	20,85
Creatinine	54	0,873 mg/dL	0,87
SGPT	54	20,92 U/L	19
SGOT	54	21,31 U/L	21

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Tabel 2 Median Stomatocyte

Stomatocytes	Number of Patients	Median (/1000 cells)
Overall	54	3
Male	37	4
Female	17	3

Table 3 Stomatocyte Range

Variable	Percentage
Total	0-1,85%
Male	0-2%
Female	0-1,7%

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DISCUSSION

The results of this study indicate that stomatocytes can be found in healthy samples at low percentages. This finding is consistent with the results of other studies. The reference ranges we obtained were as follows: an overall reference range of 0–1.85%. In men, the range was 0–2%, and in women, 0–1.7%. Our findings are comparable to those of other studies, which state that stomatocytes are found in the normal population with a reference range of 0–3%. Therefore, our results support existing data (Perrin, Ranta, and Lesesve 2015; Pozdnyakova 2025). Differences in the range of values between sexes are thought to be influenced by

hormonal factors, erythrocyte membrane structure, and ion transport regulation. These results reinforce the need for a local reference approach in interpreting erythrocyte morphology.

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Physiologically, stomatocytes are formed due to disturbances in osmotic balance and cell membrane structure, including genetic mutations (PIEZO1, KCNN4, RHAG) or exposure to certain drugs. However, there were no clinical or biochemical indications pointing to a pathological etiology in this study population (Achfidawati et al. 2019; Flatt and Bruce 2009). The 2.5–97.5% percentile method was chosen because the distribution of stomatocyte data was not normal. The use of the median and percentile range was considered more representative than the mean ± 2SD, which is susceptible to extreme values.

In clinical contexts, pathological stomatocytosis primarily includes hereditary stomatocytosis (HSt) a spectrum of hemolytic anemias due to increased cation permeability of the erythrocyte membrane, which is typically associated with hemolysis, anemia, and abnormal laboratory findings (Andolfo et al. 2025; Flatt and Bruce 2009). In addition to hereditary forms, “acquired” stomatocytes can occur in liver disease/alcohol use disorder, certain cationic drugs, and lipid metabolism disorders (Anon 2025; Wislöff and Boman 1979; Zini et al. 2021). The absence of liver/renal function abnormalities and hematologic parameters in this study supports the interpretation that the low stomatocyte frequency does not reflect an active pathological process.

Variation by Gender

The slightly higher median stomatocyte count in men (4/1000 vs. 3/1000 in women) potentially reflects physiological variations between the sexes also seen in Hb levels and erythropoietic dynamics (Bachman et al. 2014; Murphy 2014). Given the limited sample size and male predominance, this small difference should be interpreted cautiously and confirmed in a larger, more balanced sample.

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Practical Implications

Contextual Laboratory Interpretation: The presence of very low stomatocyte counts (<~2%) in individuals with a normal CBC and biochemical panel is likely physiologic/artifactual; multi-field evaluation of smears is recommended before labeling them pathological (Anon 2025). Avoid overdiagnosis of HSt: The diagnosis of HSt requires clinical correlation (hemolysis), family history, and when necessary specific testing (osmotic gradient ectacytometry) and a genetic panel (Andolfo et al. 2025). Be aware of hepatic/alcoholic comorbidities: If

stomatocytes are significantly elevated, screening for confounding factors (medication, liver function, alcohol consumption) is relevant (Wislöff and Boman 1979; Zini et al. 2021).

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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 (Anon 2025; Brihi 2024).

CONCLUSIONS

This study successfully established local reference values for stomatocyte counts in a healthy population in Jakarta and its surrounding areas using the 2.5th–97.5th percentile approach. The reference range obtained was 0–1.85% for all subjects, with an upper limit of 0–2% in men and 0–1.7% in women. The findings suggest that low stomatocyte counts are a physiological variation in healthy individuals and are not significantly associated with routine hematological parameters or liver and kidney function in the studied population. These reference values can be used as a practical guide for clinical laboratories and clinicians in Indonesia to enhance the accuracy of peripheral blood smear interpretation and prevent overdiagnosis of low stomatocyte counts.

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Reference Range of Stomatocytes in Jakarta and Surrounding Areas

Track Record Article

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Abstract

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Keyword: Stomatocytes, reference values, abnormal erythrocytes, peripheral blood smear.

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There are no lower bound and upper bound numbers in a neat format (directly 0–1.85%)

There is no clarity that the 2.5–97.5th percentile is used as a "reference interval"

INTRODUCTION

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"GAP" isn't clear enough → The last line only focuses on Indonesia, but it needs to be more explicit.

Erythrocytes play a role in transporting oxygen and carbon dioxide throughout the body's tissues. They are biconcave in structure. This shape allows them to be flexible when passing through narrow vascular spaces, but it also makes them susceptible to morphological changes. One form of erythrocyte abnormality is stomatocytes, characterized by an elliptical or lip-shaped central pallor area due to transmembrane ion imbalance or genetic mutations, such as those 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).

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Based on this, we need to establish local reference values to improve diagnostic accuracy related to changes in erythrocyte shape in Indonesia. Therefore, this study aims to determine the reference values for stomatocytes in a healthy adult population in Jakarta and its surrounding areas, and to evaluate potential differences in these values based on gender.

METHODS

This study used a descriptive cross-sectional design, and primary data were obtained through laboratory tests and peripheral blood smears. The study was conducted at the Indonesian Christian University General Hospital in Jakarta and a hospital in East Bekasi. Data collection was conducted between August 2022 and October 2024. A total of 65 samples were obtained,

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Commented [A3]: 65 → 54 final = good, but no reason was given for 11 exclusions per type exclusion.

Unclear smear reading standards: per how many fields? Which areas: tail/body/monolayer?

No interobserver QA/QC

but only 54 met the inclusion criteria. Samples were excluded due to age and laboratory results that did not meet the inclusion criteria. The target sample was adult patients undergoing medical check-ups at the two hospitals. The sampling technique used a total sampling method, so that all subjects who met the inclusion and exclusion criteria were included in the study sample. The inclusion criteria included patients aged 21–60 years, with complete laboratory test results (Hb, MCV, MCH, MCHC, RDW-CV, SGPT, SGOT, urea, creatinine) within normal limits, and no history of chronic disease or blood cell disorders. Meanwhile, exclusion criteria apply to incomplete medical record data, pregnant or breastfeeding women, a history of active smoking or alcohol consumption, and patients undergoing chemotherapy or with abnormal laboratory results.

The tools and materials used included EDTA tubes, slides, dropper pipettes, staining racks, light microscopes, and staining equipment (methanol, Wright stain, distilled water, immersion oil). The biological material was a venous blood sample from each subject. If the sample met the inclusion criteria, the study began with the preparation of a peripheral blood smear with one drop of EDTA blood taken from the vein, placed on a slide, and then the blood smear was made and fixed using methanol and Wright stain. The preparation was observed under a light microscope at 1000x magnification using immersion oil. Recording was carried out using a microscope camera. After reaching 1,000 erythrocytes, a search was conducted for stomatocytes. The researcher recorded the number of stomatocytes found. Furthermore, the examination results were verified by one clinical pathologist to ensure accuracy (Ridwan et al. 2021). Data were analyzed univariately by calculating the median and the 2.5th to 97.5th percentile range as reference values, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. Normality tests and data distribution analysis were conducted using SPSS software version 27. If the data were not normally distributed, the median was used as a measure of central tendency and the percentile range as a measure of distribution (Liana et al. 2022; Rosida and Hendriyono 2015).

RESULTS

This study involved 54 subjects, consisting of 37 men (68.52%) and 17 women (31.48%). All subjects were healthy individuals, as determined by medical check-ups and laboratory results, which included basic hematology and biochemistry parameters, and met the established inclusion and exclusion criteria. Subjects were distributed in the age range of 21–60 years.

Commented [A4]: Table 2: "Median/1000 cells" → should be "median stomatocytes per 1000 erythrocytes"

Table 3: "percentage total" → confusing; should be clear "reference interval (2.5–97.5th percentile)"

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
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Male	37	14,92 g/dL	14,1
Female	17	13,00 g/dL	12,8
MCV	54	84,49 fL	85,25
MCH	54	29,79 pg	29
MCHC	54	33,55 g/dL	33,35
RDW-SD	54	39,65 fL	39,9
RDW-CV	54	12,78 %	12,7
Urea	54	22,95 mg/dL	20,85
Creatinine	54	0,873 mg/dL	0,87
SGPT	54	20,92 U/L	19
SGOT	54	21,31 U/L	21

Tabel 2 Median Stomatocyte

Stomatocytes	Number of Patients	Median (/1000 cells)
Overall	54	3
Male	37	4
Female	17	3

Table 3 Stomatocyte Range

Variable	Percentage
Total	0-1,85%
Male	0-2%
Female	0-1,7%

DISCUSSION

The results of this study indicate that stomatocytes can be found in healthy samples at low percentages. This finding is consistent with the results of other studies. The reference ranges we obtained were as follows: an overall reference range of 0–1.85%. In men, the range was 0–2%, and in women, 0–1.7%. Our findings are comparable to those of other studies, which state that stomatocytes are found in the normal population with a reference range of 0–3%. Therefore, our results support existing data (Perrin, Ranta, and Lesesve 2015; Pozdnyakova 2025). Differences in the range of values between sexes are thought to be influenced by

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No clinical interpretation yet for cut-off versus HSt/HX threshold

No important discussion yet: pre-analytical artifacts (stomatocytes can appear as drying artifacts)

hormonal factors, erythrocyte membrane structure, and ion transport regulation. These results reinforce the need for a local reference approach in interpreting erythrocyte morphology.

Physiologically, stomatocytes are formed due to disturbances in osmotic balance and cell membrane structure, including genetic mutations (PIEZO1, KCNN4, RHAG) or exposure to certain drugs. However, there were no clinical or biochemical indications pointing to a pathological etiology in this study population (Achfidawati et al. 2019; Flatt and Bruce 2009). The 2.5–97.5% percentile method was chosen because the distribution of stomatocyte data was not normal. The use of the median and percentile range was considered more representative than the mean ± 2SD, which is susceptible to extreme values.

In clinical contexts, pathological stomatocytosis primarily includes hereditary stomatocytosis (HSt) a spectrum of hemolytic anemias due to increased cation permeability of the erythrocyte membrane, which is typically associated with hemolysis, anemia, and abnormal laboratory findings (Andolfo et al. 2025; Flatt and Bruce 2009). In addition to hereditary forms, "acquired" stomatocytes can occur in liver disease/alcohol use disorder, certain cationic drugs, and lipid metabolism disorders (Anon 2025; Wislöff and Boman 1979; Zini et al. 2021). The absence of liver/renal function abnormalities and hematologic parameters in this study supports the interpretation that the low stomatocyte frequency does not reflect an active pathological process.

Variation by Gender

The slightly higher median stomatocyte count in men (4/1000 vs. 3/1000 in women) potentially reflects physiological variations between the sexes also seen in Hb levels and erythropoietic dynamics (Bachman et al. 2014; Murphy 2014). Given the limited sample size and male predominance, this small difference should be interpreted cautiously and confirmed in a larger, more balanced sample.

Practical Implications

Contextual Laboratory Interpretation: The presence of very low stomatocyte counts (<~2%) in individuals with a normal CBC and biochemical panel is likely physiologic/artifactual; multi-field evaluation of smears is recommended before labeling them pathological (Anon 2025). Avoid overdiagnosis of HSt: The diagnosis of HSt requires clinical correlation (hemolysis), family history, and when necessary specific testing (osmotic gradient ectacytometry) and a genetic panel (Andolfo et al. 2025). Be aware of hepatic/alcoholic comorbidities: If

stomatocytes are significantly elevated, screening for confounding factors (medication, liver function, alcohol consumption) is relevant (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 (Anon 2025; Brihi 2024).

CONCLUSIONS

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This study successfully established local reference values for stomatocyte counts in a healthy population in Jakarta and its surrounding areas using the 2.5th–97.5th percentile approach. The reference range obtained was 0–1.85% for all subjects, with an upper limit of 0–2% in men and 0–1.7% in women. The findings suggest that low stomatocyte counts are a physiological variation in healthy individuals and are not significantly associated with routine hematological parameters or liver and kidney function in the studied population. These reference values can be used as a practical guide for clinical laboratories and clinicians in Indonesia to enhance the accuracy of peripheral blood smear interpretation and prevent overdiagnosis of low stomatocyte counts.

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REVIEWER

- Abstract: Concise, specific, and clearly indicates the study's focus and geographical scope.
- Abstract: Well-structured according to IMRaD format. The objective, methods, key results (0–1.85% overall range), and main conclusion are clearly stated.
- Keywords: Appropriate and relevant to the subject matter.
- Abstract: The abstract accurately summarizes the study, though it could briefly mention the key limitation (small sample size, gender imbalance) for greater balance.
- Introduction The research problem—reliance on foreign reference values and the need for local standards—is clearly defined.
- Introduction Gaps are appropriately identified: lack of Indonesian data and the influence of genetic/environmental factors.
- Introduction Literature review is generally comprehensive and up-to-date, citing key pathophysiological mechanisms and international guidelines (ICSH).
- Suggestions Introduction: The introduction could be strengthened by a more explicit statement on the *clinical consequence* of using inappropriate reference ranges (e.g., risk of over-/under-diagnosis). A brief critique of the limited existing studies on "healthy" stomatocyte prevalence would sharpen the justification.
- The primary objective (to determine local reference values) and secondary objective (to evaluate differences by gender) are clear, specific, and logically flow from the introduction.
- The research question is well-formulated and directly testable through the described methodology.
- Methodology Study Design: The descriptive cross-sectional design is appropriate for establishing reference intervals.
- Methodology Sample & Sampling: The sample size (n=54) is a major limitation, adequately acknowledged later but crucial. The total sampling

method from health check-up populations is described. Inclusion/exclusion criteria are clearly defined, though the exclusion of all active smokers/alcohol consumers creates a very "clean" but potentially non-representative "healthy" cohort.

- Methodology Variables/Indicators: Key variables (stomatocyte count, basic hematology, liver/renal function) are well-chosen. The outcome variable (stomatocytes/1000 RBCs) is standard.
- Methodology Data Collection: The peripheral blood smear protocol is described in replicable detail, including verification by a clinical pathologist, which enhances reliability.
- Methodology Data Analysis: The use of the 2.5–97.5th percentile (non-parametric) due to non-normal distribution is statistically sound and aligns with CLSI guidelines for reference intervals. The use of SPSS is standard.
- Results are presented clearly with appropriate tables (sample characteristics, median counts, reference ranges).
- Findings are thematically sound. Table 1 effectively summarizes the cohort's health status. The central findings (Table 2 & 3) are straightforward. No complex statistics were required beyond percentile derivation.
- Discussion Results are interpreted meaningfully and contextualized within existing literature (e.g., comparison to 0–3% range).
- Discussion The discussion appropriately covers physiological mechanisms, clinical implications for distinguishing pathological vs. physiological findings, and potential gender differences.
- Comparison with Literature: Good, though could be deepened by directly comparing the obtained percentiles with those from other ethnic/geographic studies if available.
- Limitations: Acknowledged transparently and thoroughly (sample size, gender imbalance, unmeasured lifestyle factors). This strengthens the manuscript's credibility.
- Conclusions are justified by the presented data.
- Implications: Practical implications for Indonesian laboratories and clinicians are well-articulated (preventing overdiagnosis). The call for larger,

more balanced studies is appropriate. A suggestion for a future multi-center Indonesian study to establish national reference ranges would strengthen the forward-looking impact.

- Reference minimum 28

Reference Range of Stomatocytes in Jakarta and Surrounding Areas

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Abstract

Stomatocytes are erythrocytes characterized by a slit-like central pallor and may appear in small numbers under physiological conditions, although increased proportions are associated with pathological states. Establishing population-specific reference intervals is essential to avoid misinterpretation of erythrocyte morphology. This study aimed to determine the reference interval for stomatocyte counts in a healthy population in Jakarta and surrounding areas and to assess their association 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 between August 2022 and October 2024. Of the 65 samples collected, 54 met the inclusion criteria. Stomatocyte counts were determined from peripheral blood smears by counting per 1,000 erythrocytes. Reference intervals were established using the 2.5th–97.5th percentile method. The reference interval for stomatocytes in the overall population was 0–1.85%. Sex-specific reference 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 emphasize the importance of using clearly defined reference intervals and clinical context when interpreting erythrocyte morphology to prevent overdiagnosis.

Keyword: Stomatocytes, reference values, abnormal erythrocytes, peripheral blood smear.

INTRODUCTION

Erythrocytes play a role in transporting oxygen and carbon dioxide throughout the body's tissues. They are biconcave in structure, a shape that allows them to be highly flexible when passing through narrow vascular spaces while maintaining a large surface area for gas exchange. However, this structural characteristic also makes erythrocytes susceptible to morphological alterations under pathological or physiological stress. One form of erythrocyte abnormality is the stomatocyte, which is characterized by an elliptical or lip-shaped central pallor resulting from disturbances in transmembrane ion balance or underlying genetic mutations, particularly involving 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). Therefore, careful evaluation of erythrocyte morphology is essential, as the presence of stomatocytes may reflect underlying membrane instability or systemic disorders and has important implications in hematological assessment.

The morphological variation of red blood cells, including stomatocytes, necessitates standardization of terminology, recognition criteria, and reporting methods to ensure interlaboratory consistency. The International Council for Standardization in Hematology (ICSH) has published guidelines for the nomenclature and grading of morphological features of peripheral blood cells, as well as updated recommendations for the quantitation of some fragment forms (e.g., schistocytes) to prevent diagnostic misinterpretation (Palmer et al. 2015; Zini et al. 2021). While the ICSH's focus on schistocyte thresholds does not directly establish stomatocyte thresholds, the spirit of standardizing morphology on blood smears provides a framework for local reporting practices and validation of low-number abnormal cell findings (Palmer et al. 2015; Zini et al. 2021).

Pathobiologically, stomatocytes can reflect impaired erythrocyte membrane permeability and cation homeostasis (Na^+/K^+) in hereditary conditions such as dehydrated hereditary stomatocytosis/hereditary xerocytosis (Andolfo, Iolascon, and Russo 2025), as well as changes in membrane lipid composition in alcohol-related liver disease, which can cause stomatocyte shapes and resolve with improvement in liver function (Ridwan, Agustina, and Makmur 2021). However, in healthy individuals, cells with stomatocyte-like morphology can also occasionally appear due to drying artifact, which is why assessment should encompass multiple smear areas and not rely on a single field of view (Chen et al. 2023). This reinforces the urgency of having local reference values for stomatocyte counts so that clinicians can differentiate physiological variations from disease manifestations.

Some references state that healthy individuals have a stomatocyte prevalence of less than 3%. In comparison, an increase in the number exceeding 5% is correlated with pathological conditions such as hereditary stomatocytosis, liver cirrhosis, fatty liver, and hemolytic anemia (Körber et al. 2017; Pozdnyakova 2025; Romanenko et al. 2024). To date, Indonesia lacks a standard reference for stomatocytes, and we still rely on data from populations abroad, such as those in America and Europe. This is because there is limited research on this topic in Indonesia. Determining normal values in Indonesia is very necessary due to the influence of genetic, ethnic, and environmental factors.

Based on this, we need to establish local reference values to improve diagnostic accuracy related to changes in erythrocyte shape in Indonesia. Therefore, this study aims to determine

the reference values for stomatocytes in a healthy adult population in Jakarta and its surrounding areas, and to evaluate potential differences in these values based on gender.

METHODS

This study employed a descriptive cross-sectional design, with primary data obtained from 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 carried out between August 2022 and October 2024. During this period, a total of 65 adult patients undergoing routine medical check-ups were initially identified and screened for eligibility. However, only 54 samples were included in the final analysis, as 11 samples were excluded after the application of predefined inclusion and exclusion criteria. Specifically, exclusions were made due to patient age falling outside the targeted range of 21–60 years and laboratory test results that did not meet the required normal reference values, which could potentially confound erythrocyte morphology assessment. The target population consisted of adult patients undergoing medical check-ups at the two hospitals, selected to represent individuals without acute or chronic conditions that may influence hematological parameters. A total sampling technique was applied, whereby all subjects who met the eligibility criteria during the study period were included to minimize selection bias and ensure comprehensive data representation. The inclusion criteria comprised patients aged 21–60 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, as well as no documented history of chronic disease or hematological disorders. Exclusion criteria included incomplete medical records, pregnancy or breastfeeding status, a history of active smoking or alcohol consumption, ongoing chemotherapy, and abnormal laboratory findings, as these factors may alter erythrocyte morphology and compromise the validity of the study results.

The tools and materials used included EDTA tubes, slides, dropper pipettes, staining racks, light microscopes, and staining equipment (methanol, Wright stain, distilled water, immersion oil). The biological material was a venous blood sample from each subject. If the sample met the inclusion criteria, the study began with the preparation of a peripheral blood smear with one drop of EDTA blood taken from the vein, placed on a slide, and then the blood smear was

made and fixed using methanol and Wright stain. The preparation was observed under a light microscope at 1000x magnification using immersion oil. Recording was carried out using a microscope camera. After reaching 1,000 erythrocytes, a search was conducted for stomatocytes. The researcher recorded the number of stomatocytes found. Furthermore, the examination results were verified by one clinical pathologist to ensure accuracy (Ridwan et al. 2021). Data were analyzed univariately by calculating the median and the 2.5th to 97.5th percentile range as reference values, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. Normality tests and data distribution analysis were conducted using SPSS software version 27. If the data were not normally distributed, the median was used as a measure of central tendency and the percentile range as a measure of distribution (Liana et al. 2022; Rosida and Hendriyono 2015).

RESULTS

This study involved 54 subjects, consisting of 37 men (68.52%) and 17 women (31.48%). All subjects were healthy individuals, as determined by medical check-ups and laboratory results, which included basic hematology and biochemistry parameters, and met the established inclusion and exclusion criteria. Subjects were distributed in the age range of 21–60 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 Sex

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 among all examined peripheral blood smears. The percentage range of 0–1.85% indicates that, in most samples, stomatocytes were not detected, and when present, they appeared only sporadically and did not exceed the upper threshold commonly considered within normal morphological variation. When stratified by sex, male subjects showed a slightly higher maximum percentage (0–2.00%) compared to female subjects (0–1.70%); however, this difference is minimal and not clinically significant. The absence of prominent stomatocytosis in the majority of samples is consistent with the strict inclusion criteria applied in this study, which selected individuals with normal hematological and biochemical parameters and no history of conditions known to affect erythrocyte membrane stability. Therefore, these findings suggest that stomatocytes observed in this population likely represent normal morphological variants rather than pathological abnormalities.

DISCUSSION

The presence of stomatocytes at low percentages in healthy individuals suggests that this erythrocyte morphology can represent a physiological variation rather than an inherent pathological condition. Stomatocytes are characterized 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—fall well within the limits reported in previous literature, which describe stomatocytes occurring in up to 3% of erythrocytes in healthy populations (Perrin et al. 2015; Pozdnyakova 2025).

The observed differences between sexes may be attributable to 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 causing clinically significant hematological abnormalities. Importantly, the low prevalence of stomatocytes observed in this study does not indicate an increased risk of future hematological disorders in the participants, as isolated stomatocytosis at minimal levels is not associated with hemolysis, anemia, or functional impairment of red blood cells. Pathological significance 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 are formed due to disturbances in osmotic balance and cell membrane structure, including genetic mutations (PIEZO1, KCNN4, RHAG) or exposure to certain drugs. However, there were no clinical or biochemical indications pointing to a pathological etiology in this study population (Achfidawati et al. 2019; Flatt and Bruce 2009). The 2.5–97.5% percentile method was chosen because the distribution of stomatocyte data was not normal. The use of the median and percentile range was considered more representative than the mean ± 2SD, which is susceptible to extreme values.

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Variation by Gender

The slightly higher median stomatocyte count observed in men (4/1000 erythrocytes) compared with women (3/1000 erythrocytes) may reflect sex-related physiological differences in erythropoiesis and red blood cell turnover, which are also known to influence hemoglobin concentration and hematocrit values (Bachman et al. 2014; Murphy 2014). Androgen hormones, particularly testosterone, stimulate erythropoietin production and enhance erythroid

progenitor proliferation, leading to increased red blood cell mass and potentially greater variability in erythrocyte morphology. In contrast, estrogen has been shown to exert stabilizing effects on erythrocyte membrane composition and oxidative balance, which may contribute to slightly lower morphological variation in women.

Additionally, sex-specific differences in erythrocyte membrane lipid composition, intracellular ion transport, and cell hydration status may influence the tendency toward transient stomatocytic shape changes without pathological significance. These subtle morphological variations are generally considered 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 the present study, the observed difference may also be influenced by sampling variability rather than a true biological effect. Consequently, this finding should be interpreted with caution and warrants confirmation in future studies involving larger cohorts with balanced sex distribution and controlled assessment of potential confounders such as age, hydration status, and pre-analytical factor.

Practical Implications

Contextual Laboratory Interpretation. The detection 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 true pathology. Factors such as smear preparation technique, drying time, anticoagulant effects, and field selection during microscopic evaluation can influence erythrocyte morphology and may lead to the incidental appearance of stomatocytes. Therefore, assessment across multiple microscopic fields and, when necessary, repeat smear examination are essential to ensure accurate interpretation before classifying such findings as abnormal (Chen et al. 2023).

Avoidance of Overdiagnosis of Hereditary Stomatocytosis (HSt). The presence of stomatocytes alone, particularly at low frequencies, is insufficient for diagnosing hereditary stomatocytosis. HSt is a rare hemolytic disorder characterized by persistent stomatocytosis accompanied by clinical and laboratory evidence of hemolysis, including anemia, reticulocytosis, elevated lactate dehydrogenase, and reduced haptoglobin levels. A thorough clinical evaluation, detailed family history, and correlation with hemolytic markers are critical components of diagnosis. In cases where clinical suspicion remains, advanced diagnostic modalities such as osmotic gradient ectacytometry and targeted genetic testing for membrane

protein or ion channel mutations are required to establish a definitive diagnosis (Andolfo 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 (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 sex-specific upper limits of 2.0% in men and 1.7% in women. The absence of significant associations between stomatocyte counts and routine hematological parameters or liver and kidney function supports the interpretation that low-level stomatocytosis is not indicative of underlying pathology. The use of these locally derived reference intervals, together with appropriate clinical correlation, is essential to improve peripheral blood smear interpretation and to reduce the risk of overdiagnosis in routine laboratory practice.

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Reference Range of Stomatocytes in Jakarta and Surrounding Areas

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Abstract

Stomatocytes are a type of abnormal erythrocyte characterized by an elliptical or cleft lip-like central pallor. Physiologically, stomatocytes can occur in minimal numbers; however, an increase in their number is often associated with pathological conditions, such as liver disease, hemolytic anemia, and certain genetic disorders. Determining local reference values for stomatocyte counts in healthy populations is crucial for distinguishing normal variations from pathological conditions, particularly since geographic, genetic, and environmental factors can influence hematological variation between populations. This study aims to determine the reference value of stomatocyte count in a healthy population in Jakarta and its surroundings, and to evaluate its relationship with basic hematological parameters, liver function, and kidney function. This research is a descriptive study with a cross-sectional design. Samples were taken consecutively from healthy individuals undergoing health check-ups at UKI General Hospital and East Bekasi Hospital between August 2022 and October 2024. Of the 65 samples, 54 subjects met the inclusion criteria, namely healthy individuals without a history of hematological, hepatic, or renal disease. Peripheral blood smear examination was performed to calculate the number of stomatocytes per 1000 erythrocytes. Data analysis used SPSS v.27 software with a 2.5–97.5% percentile distribution approach to determine reference values. The reference value for stomatocytes in a healthy population as a whole is 0–1.85%. Based on gender, the reference value for men is 0–2%, while for women it is 0–1.7%. Further analysis showed that the stomatocyte count was not significantly associated with routine hematological parameters, liver function, or kidney function. Stomatocytes can be found in small numbers in healthy individuals, with reference values ranging from 0–1.85% in the population of Jakarta and its surrounding areas. These findings support the need to consider the clinical context and local reference values for interpreting erythrocyte morphology.

Keyword: Stomatocytes, reference values, abnormal erythrocytes, peripheral blood smear.

Commented [A1]: Too long for an abstract

There are no lower bound and upper bound numbers in a neat format (directly 0–1.85%)

There is no clarity that the 2.5–97.5th percentile is used as a "reference interval"

INTRODUCTION

Commented [A2]: Too much of a literature review on pathobiology → OK for discussion, not an intro.

"GAP" isn't clear enough → The last line only focuses on Indonesia, but it needs to be more explicit.

Erythrocytes play a role in transporting oxygen and carbon dioxide throughout the body's tissues. They are biconcave in structure. This shape allows them to be flexible when passing through narrow vascular spaces, but it also makes them susceptible to morphological changes. One form of erythrocyte abnormality is stomatocytes, characterized by an elliptical or lip-shaped central pallor area due to transmembrane ion imbalance or genetic mutations, such as those 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).

The morphological variation of red blood cells, including stomatocytes, necessitates standardization of terminology, recognition criteria, and reporting methods to ensure interlaboratory consistency. The International Council for Standardization in Hematology (ICSH) has published guidelines for the nomenclature and grading of morphological features of peripheral blood cells, as well as updated recommendations for the quantitation of some

fragment forms (e.g., schistocytes) to prevent diagnostic misinterpretation (Palmer et al. 2015; Zini et al. 2021). While the ICSH's focus on schistocyte thresholds does not directly establish stomatocyte thresholds, the spirit of standardizing morphology on blood smears provides a framework for local reporting practices and validation of low-number abnormal cell findings (Palmer et al. 2015; Zini et al. 2021).

Pathobiologically, stomatocytes can reflect impaired erythrocyte membrane permeability and cation homeostasis (Na^+/K^+) in hereditary conditions such as dehydrated hereditary stomatocytosis/hereditary xerocytosis (Andolfo, Iolascon, and Russo 2025), as well as changes in membrane lipid composition in alcohol-related liver disease, which can cause stomatocyte shapes and resolve with improvement in liver function (Ridwan, Agustina, and Makmur 2021). However, in healthy individuals, cells with stomatocyte-like morphology can also occasionally appear due to drying artifact, which is why assessment should encompass multiple smear areas and not rely on a single field of view (Anon 2025). This reinforces the urgency of having local reference values for stomatocyte counts so that clinicians can differentiate physiological variations from disease manifestations.

Some references state that healthy individuals have a stomatocyte prevalence of less than 3%. In comparison, an increase in the number exceeding 5% is correlated with pathological conditions such as hereditary stomatocytosis, liver cirrhosis, fatty liver, and hemolytic anemia (Körber et al. 2017; Pozdnyakova 2025; Romanenko et al. 2024). To date, Indonesia lacks a standard reference for stomatocytes, and we still rely on data from populations abroad, such as those in America and Europe. This is because there is limited research on this topic in Indonesia. Determining normal values in Indonesia is very necessary due to the influence of genetic, ethnic, and environmental factors.

Based on this, we need to establish local reference values to improve diagnostic accuracy related to changes in erythrocyte shape in Indonesia. Therefore, this study aims to determine the reference values for stomatocytes in a healthy adult population in Jakarta and its surrounding areas, and to evaluate potential differences in these values based on gender.

METHODS

This study used a descriptive cross-sectional design, and primary data were obtained through laboratory tests and peripheral blood smears. The study was conducted at the Indonesian Christian University General Hospital in Jakarta and a hospital in East Bekasi. Data collection was conducted between August 2022 and October 2024. A total of 65 samples were obtained,

Page PAGE 1* MERGEFORMAT

Commented [A3]: 65 → 54 final = good, but no reason was given for 11 exclusions per type exclusion.

Unclear smear reading standards: per how many fields? Which areas: tail/body/monolayer?

No interobserver QA/QC

but only 54 met the inclusion criteria. Samples were excluded due to age and laboratory results that did not meet the inclusion criteria. The target sample was adult patients undergoing medical check-ups at the two hospitals. The sampling technique used a total sampling method, so that all subjects who met the inclusion and exclusion criteria were included in the study sample. The inclusion criteria included patients aged 21–60 years, with complete laboratory test results (Hb, MCV, MCH, MCHC, RDW-CV, SGPT, SGOT, urea, creatinine) within normal limits, and no history of chronic disease or blood cell disorders. Meanwhile, exclusion criteria apply to incomplete medical record data, pregnant or breastfeeding women, a history of active smoking or alcohol consumption, and patients undergoing chemotherapy or with abnormal laboratory results.

The tools and materials used included EDTA tubes, slides, dropper pipettes, staining racks, light microscopes, and staining equipment (methanol, Wright stain, distilled water, immersion oil). The biological material was a venous blood sample from each subject. If the sample met the inclusion criteria, the study began with the preparation of a peripheral blood smear with one drop of EDTA blood taken from the vein, placed on a slide, and then the blood smear was made and fixed using methanol and Wright stain. The preparation was observed under a light microscope at 1000x magnification using immersion oil. Recording was carried out using a microscope camera. After reaching 1,000 erythrocytes, a search was conducted for stomatocytes. The researcher recorded the number of stomatocytes found. Furthermore, the examination results were verified by one clinical pathologist to ensure accuracy (Ridwan et al. 2021). Data were analyzed univariately by calculating the median and the 2.5th to 97.5th percentile range as reference values, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. Normality tests and data distribution analysis were conducted using SPSS software version 27. If the data were not normally distributed, the median was used as a measure of central tendency and the percentile range as a measure of distribution (Liana et al. 2022; Rosida and Hendriyono 2015).

RESULTS

This study involved 54 subjects, consisting of 37 men (68.52%) and 17 women (31.48%). All subjects were healthy individuals, as determined by medical check-ups and laboratory results, which included basic hematology and biochemistry parameters, and met the established inclusion and exclusion criteria. Subjects were distributed in the age range of 21–60 years.

Commented [A4]: Table 2: "Median/1000 cells" → should be "median stomatocytes per 1000 erythrocytes"

Table 3: "percentage total" → confusing; should be clear "reference interval (2.5–97.5th percentile)"

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			
Male	37	14,92 g/dL	14,1
Female	17	13,00 g/dL	12,8
MCV	54	84,49 fL	85,25
MCH	54	29,79 pg	29
MCHC	54	33,55 g/dL	33,35
RDW-SD	54	39,65 fL	39,9
RDW-CV	54	12,78 %	12,7
Urea	54	22,95 mg/dL	20,85
Creatinine	54	0,873 mg/dL	0,87
SGPT	54	20,92 U/L	19
SGOT	54	21,31 U/L	21

Tabel 2 Median Stomatocyte

Stomatocytes	Number of Patients	Median (/1000 cells)
Overall	54	3
Male	37	4
Female	17	3

Table 3 Stomatocyte Range

Variable	Percentage
Total	0-1,85%
Male	0-2%
Female	0-1,7%

DISCUSSION

The results of this study indicate that stomatocytes can be found in healthy samples at low percentages. This finding is consistent with the results of other studies. The reference ranges we obtained were as follows: an overall reference range of 0–1.85%. In men, the range was 0–2%, and in women, 0–1.7%. Our findings are comparable to those of other studies, which state that stomatocytes are found in the normal population with a reference range of 0–3%. Therefore, our results support existing data (Perrin, Ranta, and Lesesve 2015; Pozdnyakova 2025). Differences in the range of values between sexes are thought to be influenced by

Commented [A5]: Mostly repeating background

No clinical interpretation yet for cut-off versus HSt/HX threshold

No important discussion yet: pre-analytical artifacts (stomatocytes can appear as drying artifacts)

hormonal factors, erythrocyte membrane structure, and ion transport regulation. These results reinforce the need for a local reference approach in interpreting erythrocyte morphology.

Physiologically, stomatocytes are formed due to disturbances in osmotic balance and cell membrane structure, including genetic mutations (PIEZO1, KCNN4, RHAG) or exposure to certain drugs. However, there were no clinical or biochemical indications pointing to a pathological etiology in this study population (Achfidawati et al. 2019; Flatt and Bruce 2009). The 2.5–97.5% percentile method was chosen because the distribution of stomatocyte data was not normal. The use of the median and percentile range was considered more representative than the mean ± 2SD, which is susceptible to extreme values.

In clinical contexts, pathological stomatocytosis primarily includes hereditary stomatocytosis (HSt) a spectrum of hemolytic anemias due to increased cation permeability of the erythrocyte membrane, which is typically associated with hemolysis, anemia, and abnormal laboratory findings (Andolfo et al. 2025; Flatt and Bruce 2009). In addition to hereditary forms, "acquired" stomatocytes can occur in liver disease/alcohol use disorder, certain cationic drugs, and lipid metabolism disorders (Anon 2025; Wislöff and Boman 1979; Zini et al. 2021). The absence of liver/renal function abnormalities and hematologic parameters in this study supports the interpretation that the low stomatocyte frequency does not reflect an active pathological process.

Variation by Gender

The slightly higher median stomatocyte count in men (4/1000 vs. 3/1000 in women) potentially reflects physiological variations between the sexes also seen in Hb levels and erythropoietic dynamics (Bachman et al. 2014; Murphy 2014). Given the limited sample size and male predominance, this small difference should be interpreted cautiously and confirmed in a larger, more balanced sample.

Practical Implications

Contextual Laboratory Interpretation: The presence of very low stomatocyte counts (<~2%) in individuals with a normal CBC and biochemical panel is likely physiologic/artifactual; multi-field evaluation of smears is recommended before labeling them pathological (Anon 2025). Avoid overdiagnosis of HSt: The diagnosis of HSt requires clinical correlation (hemolysis), family history, and when necessary specific testing (osmotic gradient ectacytometry) and a genetic panel (Andolfo et al. 2025). Be aware of hepatic/alcoholic comorbidities: If

stomatocytes are significantly elevated, screening for confounding factors (medication, liver function, alcohol consumption) is relevant (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 (Anon 2025; Brihi 2024).

CONCLUSIONS

Commented [A6]: only restatement results

This study successfully established local reference values for stomatocyte counts in a healthy population in Jakarta and its surrounding areas using the 2.5th–97.5th percentile approach. The reference range obtained was 0–1.85% for all subjects, with an upper limit of 0–2% in men and 0–1.7% in women. The findings suggest that low stomatocyte counts are a physiological variation in healthy individuals and are not significantly associated with routine hematological parameters or liver and kidney function in the studied population. These reference values can be used as a practical guide for clinical laboratories and clinicians in Indonesia to enhance the accuracy of peripheral blood smear interpretation and prevent overdiagnosis of low stomatocyte counts.

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Reference Range of Stomatocytes in Jakarta and Surrounding Areas

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Abstract

Stomatocytes are erythrocytes distinguished by a slit-like central pallor. While they may occur in small numbers under physiological conditions, elevated proportions are linked to pathological states. Establishing population-specific reference intervals is crucial to prevent misinterpretation of erythrocyte morphology. This study determined the reference interval for stomatocyte counts in a healthy population from Jakarta and surrounding areas and evaluated their association 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 assessed from peripheral blood smears by counting per 1,000 erythrocytes. Reference intervals were calculated using the 2.5th to 97.5th percentile method. The reference interval for stomatocytes in the overall population was 0 to 1.85%. Sex-specific reference intervals were 0 to 2.0% in men and 0 to 1.7% in women. No significant associations were found between stomatocyte counts and complete blood count parameters, liver function tests, or kidney function tests. In summary, stomatocytes may be present at low levels in healthy individuals, with a locally derived reference interval of 0 to 1.85%. These results underscore the necessity of applying clearly defined reference intervals and considering clinical context when interpreting erythrocyte morphology to avoid overdiagnosis.

Keyword: Stomatocytes, reference values, abnormal erythrocytes, peripheral blood smear.

INTRODUCTION

Erythrocytes transport oxygen and carbon dioxide throughout the body's tissues. Their biconcave structure confers high flexibility, enabling passage through narrow vascular spaces and providing a large surface area for gas exchange. This structural feature, however, also renders erythrocytes vulnerable to morphological changes under pathological or physiological stress. Stomatocytes represent one such abnormality, characterized by an elliptical or lip-shaped central pallor arising from disruptions 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 essential

because the presence of stomatocytes may indicate underlying membrane instability or systemic disorders and carries significant implications for hematological assessment.

The morphological variation of red blood cells, including stomatocytes, necessitates standardization of terminology, recognition criteria, and reporting methods to ensure interlaboratory consistency. The International Council for Standardization in Hematology (ICSH) has published guidelines for the nomenclature and grading of morphological features of peripheral blood cells, as well as updated recommendations for the quantitation of some fragment forms (e.g., schistocytes) to prevent diagnostic misinterpretation (Palmer et al. 2015; Zini et al. 2021). While the ICSH's focus on schistocyte thresholds does not directly establish stomatocyte thresholds, the spirit of standardizing morphology on blood smears provides a framework for local reporting practices and validation of low-number abnormal cell findings (Palmer et al. 2015; Zini et al. 2021).

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Based on this, we need to establish local reference values to improve diagnostic accuracy related to changes in erythrocyte shape in Indonesia. Therefore, this study aims to determine

the reference values for stomatocytes in a healthy adult population in Jakarta and its surrounding areas, and to evaluate potential differences in these values based on gender.

METHODS

A descriptive cross-sectional design was utilized, with primary data collected through laboratory examinations and peripheral blood smear analyses. The research 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 timeframe, 65 adult patients undergoing routine medical check-ups were initially identified and screened for eligibility. Of these, 54 samples were included in the final analysis following the application of predefined inclusion and exclusion criteria. Eleven samples were excluded due to patient age outside the targeted range of 21 to 60 years or laboratory test results that did not meet the required normal reference values, which could confound erythrocyte morphology assessment. The target population comprised adult patients 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 employed, including all subjects who met the eligibility criteria during the study period to minimize selection bias and ensure comprehensive data representation. Inclusion criteria required patients 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 encompassed incomplete medical records, pregnancy or breastfeeding, a history of 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 included EDTA tubes, slides, dropper pipettes, staining racks, light microscopes, and staining equipment (methanol, Wright stain, distilled water, immersion oil). The biological material was a venous blood sample from each subject. If the sample met the inclusion criteria, the study began with the preparation of a peripheral blood smear with one drop of EDTA blood taken from the vein, placed on a slide, and then the blood smear was made and fixed using methanol and Wright stain. The preparation was observed under a light

microscope at 1000x magnification using immersion oil. Recording was carried out using a microscope camera. After reaching 1,000 erythrocytes, a search was conducted for stomatocytes. The researcher recorded the number of stomatocytes found. Furthermore, the examination results were verified by one clinical pathologist to ensure accuracy (Ridwan et al. 2021). Data were analyzed univariately by calculating the median and the 2.5th to 97.5th percentile range as reference values, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. Normality tests and data distribution analysis were conducted using SPSS software version 27. If the data were not normally distributed, the median was used as a measure of central tendency and the percentile range as a measure of distribution (Liana et al. 2022; Rosida and Hendriyono 2015).

RESULTS

This study involved 54 subjects, consisting of 37 men (68.52%) and 17 women (31.48%). All subjects were healthy individuals, as determined by medical check-ups and laboratory results, which included basic hematology and biochemistry parameters, and met the established inclusion and exclusion criteria. Subjects were distributed in the age range of 21–60 years.

Table 1 Sample Data Distribution (n=54)

Parameter	Subject	Mean	Median
Age			
21–30 years	11 (20,75%)	23,55	24
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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
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Red Cell Distribution Width–Standard Deviation (RDW-SD)	54	39,65 fL	39,9
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Tabel 2 Median Stomatocyte

Stomatocytes	Number of Patients	Median Stomatocytes per 1000 Erythrocytes
Overall	54	3
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Table 3 Percentage of Stomatocyte Findings by Sex

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 among all examined peripheral blood smears. The percentage range of 0–1.85% indicates that, in most samples, stomatocytes were not detected, and when present, they appeared only sporadically and did not exceed the upper threshold commonly considered within normal morphological variation. When stratified by sex, male subjects showed a slightly higher maximum percentage (0–2.00%) compared to female subjects (0–1.70%); however, this difference is minimal and not clinically significant. The absence of prominent stomatocytosis in the majority of samples is consistent with the strict inclusion criteria applied in this study, which selected individuals with normal hematological and biochemical parameters and no history of conditions known to affect erythrocyte membrane stability. Therefore, these findings suggest that stomatocytes observed in this population likely represent normal morphological variants rather than pathological abnormalities.

DISCUSSION

The presence of stomatocytes at low percentages in healthy individuals suggests that this erythrocyte morphology can represent a physiological variation rather than an inherent pathological condition. Stomatocytes are characterized 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, fall well within the limits reported in previous literature, which describe stomatocytes occurring in up

to 3% of erythrocytes in healthy populations (Perrin et al. 2015; Pozdnyakova 2025). The observed differences between sexes may be attributable to 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 causing clinically significant hematological abnormalities. Importantly, the low prevalence of stomatocytes observed in this study does not indicate an increased risk of future hematological disorders in the participants, as isolated stomatocytosis at minimal levels is not associated with hemolysis, anemia, or functional impairment of red blood cells. Pathological significance 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 are formed due to disturbances in osmotic balance and cell membrane structure, including genetic mutations (PIEZO1, KCNN4, RHAG) or exposure to certain drugs. However, there were no clinical or biochemical indications pointing to a pathological etiology in this study population (Achfidawati et al. 2019; Flatt and Bruce 2009). The 2.5–97.5% percentile method was chosen because the distribution of stomatocyte data was not normal. The use of the median and percentile range was considered more representative than the mean ± 2SD, which is susceptible to extreme values.

In clinical contexts, pathological stomatocytosis primarily includes hereditary stomatocytosis (HSt) a spectrum of hemolytic anemias due to increased cation permeability of the erythrocyte membrane, which is typically associated with hemolysis, anemia, and abnormal laboratory findings (Andolfo et al. 2025; Flatt and Bruce 2009). In addition to hereditary forms, “acquired” stomatocytes can occur in liver disease/alcohol use disorder, certain cationic drugs, and lipid metabolism disorders (Wislöff and Boman 1979; Zini et al. 2021). The absence of liver/renal function abnormalities and hematologic parameters in this study supports the interpretation that the low stomatocyte frequency does not reflect an active pathological process.

Variation by Gender

The slightly higher median stomatocyte count observed in men (4/1000 erythrocytes) compared with women (3/1000 erythrocytes) may reflect sex-related physiological differences in erythropoiesis and red blood cell turnover, which are also known to influence hemoglobin concentration and hematocrit values (Bachman et al. 2014; Murphy 2014). Androgen

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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 due to physiological variation or pre-analytical and analytical artifacts, rather than to underlying pathology. Variables such as smear preparation technique, drying time, anticoagulant effects, and field selection during microscopic evaluation can affect erythrocyte morphology and lead to the incidental observation of stomatocytes. Consequently, evaluation across multiple microscopic fields and, when indicated, repeat smear examination are necessary 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, especially at low frequencies, is insufficient for the diagnosis of hereditary stomatocytosis. Hereditary stomatocytosis is a rare hemolytic disorder defined 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).

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 transportmechanisms. 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 (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 sex-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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Reference Range of Stomatocytes in Jakarta and Surrounding Areas

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Abstract

Stomatocytes are erythrocytes distinquished by a slit-like central pallor. While they may occur in small numbers under physiological conditions, elevated proportions are linked to pathological states. Establishing population-specific reference intervals is crucial to prevent misinterpretation of erythrocyte morphology. This study determined the reference interval for stomatocyte counts in a healthy population from Jakarta and surrounding areas and evaluated their association 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 assessed from peripheral blood smears by counting per 1,000 erythrocytes. Reference intervals were calculated using the 2.5th to 97.5th percentile method. The reference interval for stomatocytes in the overall population was 0 to 1.85%. Sex-specific reference intervals were 0 to 2.0% in men and 0 to 1.7% in women. No significant associations were found between stomatocyte counts and complete blood count parameters, liver function tests, or kidney function tests. In summary, stomatocytes may be present at low levels in healthy individuals, with a locally derived reference interval of 0 to 1.85%. These results underscore the necessity of applying clearly defined reference intervals and considering clinical context when interpreting erythrocyte morphology to avoid overdiagnosis.

Keyword: Stomatocytes, reference values, abnormal erythrocytes, peripheral blood smear.

INTRODUCTION

Erythrocytes transport oxygen and carbon dioxide throughout the body's tissues. Their biconcave structure confers high flexibility, enabling passage through narrow vascular spaces and providing a large surface area for gas exchange. This structural feature, however, also renders erythrocytes vulnerable to morphological changes under pathological or physiological stress. Stomatocytes represent one such abnormality, characterized by an elliptical or lip-shaped central pallor arising from disruptions 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 essential

because the presence of stomatocytes may indicate underlying membrane instability or systemic disorders and carries significant implications for hematological assessment.

The morphological variation of red blood cells, including stomatocytes, necessitates standardization of terminology, recognition criteria, and reporting methods to ensure interlaboratory consistency. The International Council for Standardization in Hematology (ICSH) has published guidelines for the nomenclature and grading of morphological features of peripheral blood cells, as well as updated recommendations for the quantitation of some fragment forms (e.g., schistocytes) to prevent diagnostic misinterpretation (Palmer et al. 2015; Zini et al. 2021). While the ICSH's focus on schistocyte thresholds does not directly establish stomatocyte thresholds, the spirit of standardizing morphology on blood smears provides a framework for local reporting practices and validation of low-number abnormal cell findings (Palmer et al. 2015; Zini et al. 2021).

Pathobiologically, stomatocytes can reflect impaired erythrocyte membrane permeability and cation homeostasis (Na^+/K^+) in hereditary conditions such as dehydrated hereditary stomatocytosis/hereditary xerocytosis (Andolfo, Iolascon, and Russo 2025), as well as changes in membrane lipid composition in alcohol-related liver disease, which can cause stomatocyte shapes and resolve with improvement in liver function (Ridwan, Agustina, and Makmur 2021). However, in healthy individuals, cells with stomatocyte-like morphology can also occasionally appear due to drying artifact, which is why assessment should encompass multiple smear areas and not rely on a single field of view (Chen et al. 2023). This reinforces the urgency of having local reference values for stomatocyte counts so that clinicians can differentiate physiological variations from disease manifestations.

Some references state that healthy individuals have a stomatocyte prevalence of less than 3%. In comparison, an increase in the number exceeding 5% is correlated with pathological conditions such as hereditary stomatocytosis, liver cirrhosis, fatty liver, and hemolytic anemia (Körber et al. 2017; Pozdnyakova 2025; Romanenko et al. 2024). To date, Indonesia lacks a standard reference for stomatocytes, and we still rely on data from populations abroad, such as those in America and Europe. This is because there is limited research on this topic in Indonesia. Determining normal values in Indonesia is very necessary due to the influence of genetic, ethnic, and environmental factors.

Based on this, we need to establish local reference values to improve diagnostic accuracy related to changes in erythrocyte shape in Indonesia. Therefore, this study aims to determine

the reference values for stomatocytes in a healthy adult population in Jakarta and its surrounding areas, and to evaluate potential differences in these values based on gender.

METHODS

A descriptive cross-sectional design was utilized, with primary data collected through laboratory examinations and peripheral blood smear analyses. The research 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 timeframe, 65 adult patients undergoing routine medical check-ups were initially identified and screened for eligibility. Of these, 54 samples were included in the final analysis following the application of predefined inclusion and exclusion criteria. Eleven samples were excluded due to patient age outside the targeted range of 21 to 60 years or laboratory test results that did not meet the required normal reference values, which could confound erythrocyte morphology assessment. The target population comprised adult patients 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 employed, including all subjects who met the eligibility criteria during the study period to minimize selection bias and ensure comprehensive data representation. Inclusion criteria required patients 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 encompassed incomplete medical records, pregnancy or breastfeeding, a history of 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.

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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 due to physiological variation or pre-analytical and analytical artifacts, rather than to underlying pathology. Variables such as smear preparation technique, drying time, anticoagulant effects, and field selection during microscopic evaluation can affect erythrocyte morphology and lead to the incidental observation of stomatocytes. Consequently, evaluation across multiple microscopic fields and, when indicated, repeat smear examination are necessary 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, especially at low frequencies, is insufficient for the diagnosis of hereditary stomatocytosis. Hereditary stomatocytosis is a rare hemolytic disorder defined 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

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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 sex-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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Reference Range of Stomatocytes in Jakarta and Surrounding Areas

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Abstract

Stomatocytes are erythrocytes distinquished by a slit-like central pallor. While they may occur in small numbers under physiological conditions, elevated proportions are linked to pathological states. Establishing population-specific reference intervals is crucial to prevent misinterpretation of erythrocyte morphology. This study determined the reference interval for stomatocyte counts in a healthy population from Jakarta and surrounding areas and evaluated their association 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 assessed from peripheral blood smears by counting per 1,000 erythrocytes. Reference intervals were calculated using the 2.5th to 97.5th percentile method. The reference interval for stomatocytes in the overall population was 0 to 1.85%. Sex-specific reference intervals were 0 to 2.0% in men and 0 to 1.7% in women. No significant associations were found between stomatocyte counts and complete blood count parameters, liver function tests, or kidney function tests. In summary, stomatocytes may be present at low levels in healthy individuals, with a locally derived reference interval of 0 to 1.85%. These results underscore the necessity of applying clearly defined reference intervals and considering clinical context when interpreting erythrocyte morphology to avoid overdiagnosis.

Keyword: Stomatocytes, reference values, abnormal erythrocytes, peripheral blood smear.

INTRODUCTION

Erythrocytes transport oxygen and carbon dioxide throughout the body's tissues. Their biconcave structure confers high flexibility, enabling passage through narrow vascular spaces and providing a large surface area for gas exchange. This structural feature, however, also renders erythrocytes vulnerable to morphological changes under pathological or physiological stress. Stomatocytes represent one such abnormality, characterized by an elliptical or lip-shaped central pallor arising from disruptions 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 essential

because the presence of stomatocytes may indicate underlying membrane instability or systemic disorders and carries significant implications for hematological assessment.

The morphological variation of red blood cells, including stomatocytes, necessitates standardization of terminology, recognition criteria, and reporting methods to ensure interlaboratory consistency. The International Council for Standardization in Hematology (ICSH) has published guidelines for the nomenclature and grading of morphological features of peripheral blood cells, as well as updated recommendations for the quantitation of some fragment forms (e.g., schistocytes) to prevent diagnostic misinterpretation (Palmer et al. 2015; Zini et al. 2021). While the ICSH's focus on schistocyte thresholds does not directly establish stomatocyte thresholds, the spirit of standardizing morphology on blood smears provides a framework for local reporting practices and validation of low-number abnormal cell findings (Palmer et al. 2015; Zini et al. 2021).

Pathobiologically, stomatocytes can reflect impaired erythrocyte membrane permeability and cation homeostasis (Na^+/K^+) in hereditary conditions such as dehydrated hereditary stomatocytosis/hereditary xerocytosis (Andolfo, Iolascon, and Russo 2025), as well as changes in membrane lipid composition in alcohol-related liver disease, which can cause stomatocyte shapes and resolve with improvement in liver function (Ridwan, Agustina, and Makmur 2021). However, in healthy individuals, cells with stomatocyte-like morphology can also occasionally appear due to drying artifact, which is why assessment should encompass multiple smear areas and not rely on a single field of view (Chen et al. 2023). This reinforces the urgency of having local reference values for stomatocyte counts so that clinicians can differentiate physiological variations from disease manifestations.

Some references state that healthy individuals have a stomatocyte prevalence of less than 3%. In comparison, an increase in the number exceeding 5% is correlated with pathological conditions such as hereditary stomatocytosis, liver cirrhosis, fatty liver, and hemolytic anemia (Körber et al. 2017; Pozdnyakova 2025; Romanenko et al. 2024). To date, Indonesia lacks a standard reference for stomatocytes, and we still rely on data from populations abroad, such as those in America and Europe. This is because there is limited research on this topic in Indonesia. Determining normal values in Indonesia is very necessary due to the influence of genetic, ethnic, and environmental factors.

Based on this, we need to establish local reference values to improve diagnostic accuracy related to changes in erythrocyte shape in Indonesia. Therefore, this study aims to determine

the reference values for stomatocytes in a healthy adult population in Jakarta and its surrounding areas, and to evaluate potential differences in these values based on gender.

METHODS

A descriptive cross-sectional design was utilized, with primary data collected through laboratory examinations and peripheral blood smear analyses. The research 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 timeframe, 65 adult patients undergoing routine medical check-ups were initially identified and screened for eligibility. Of these, 54 samples were included in the final analysis following the application of predefined inclusion and exclusion criteria. Eleven samples were excluded due to patient age outside the targeted range of 21 to 60 years or laboratory test results that did not meet the required normal reference values, which could confound erythrocyte morphology assessment. The target population comprised adult patients 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 employed, including all subjects who met the eligibility criteria during the study period to minimize selection bias and ensure comprehensive data representation. Inclusion criteria required patients 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 encompassed incomplete medical records, pregnancy or breastfeeding, a history of 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 included EDTA tubes, slides, dropper pipettes, staining racks, light microscopes, and staining equipment (methanol, Wright stain, distilled water, immersion oil). The biological material was a venous blood sample from each subject. If the sample met the inclusion criteria, the study began with the preparation of a peripheral blood smear with one drop of EDTA blood taken from the vein, placed on a slide, and then the blood smear was made and fixed using methanol and Wright stain. The preparation was observed under a light

microscope at 1000x magnification using immersion oil. Recording was carried out using a microscope camera. After reaching 1,000 erythrocytes, a search was conducted for stomatocytes. The researcher recorded the number of stomatocytes found. Furthermore, the examination results were verified by one clinical pathologist to ensure accuracy (Ridwan et al. 2021). Data were analyzed univariately by calculating the median and the 2.5th to 97.5th percentile range as reference values, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. Normality tests and data distribution analysis were conducted using SPSS software version 27. If the data were not normally distributed, the median was used as a measure of central tendency and the percentile range as a measure of distribution (Liana et al. 2022; Rosida and Hendriyono 2015).

RESULTS

This study involved 54 subjects, consisting of 37 men (68.52%) and 17 women (31.48%). All subjects were healthy individuals, as determined by medical check-ups and laboratory results, which included basic hematology and biochemistry parameters, and met the established inclusion and exclusion criteria. Subjects were distributed in the age range of 21–60 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 Sex

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 among all examined peripheral blood smears. The percentage range of 0–1.85% indicates that, in most samples, stomatocytes were not detected, and when present, they appeared only sporadically and did not exceed the upper threshold commonly considered within normal morphological variation. When stratified by sex, male subjects showed a slightly higher maximum percentage (0–2.00%) compared to female subjects (0–1.70%); however, this difference is minimal and not clinically significant. The absence of prominent stomatocytosis in the majority of samples is consistent with the strict inclusion criteria applied in this study, which selected individuals with normal hematological and biochemical parameters and no history of conditions known to affect erythrocyte membrane stability. Therefore, these findings suggest that stomatocytes observed in this population likely represent normal morphological variants rather than pathological abnormalities.

DISCUSSION

The presence of stomatocytes at low percentages in healthy individuals suggests that this erythrocyte morphology can represent a physiological variation rather than an inherent pathological condition. Stomatocytes are characterized 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, fall well within the limits reported in previous literature, which describe stomatocytes occurring in up

to 3% of erythrocytes in healthy populations (Perrin et al. 2015; Pozdnyakova 2025). The observed differences between sexes may be attributable to 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 causing clinically significant hematological abnormalities. Importantly, the low prevalence of stomatocytes observed in this study does not indicate an increased risk of future hematological disorders in the participants, as isolated stomatocytosis at minimal levels is not associated with hemolysis, anemia, or functional impairment of red blood cells. Pathological significance 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 are formed due to disturbances in osmotic balance and cell membrane structure, including genetic mutations (PIEZO1, KCNN4, RHAG) or exposure to certain drugs. However, there were no clinical or biochemical indications pointing to a pathological etiology in this study population (Achfidawati et al. 2019; Flatt and Bruce 2009). The 2.5–97.5% percentile method was chosen because the distribution of stomatocyte data was not normal. The use of the median and percentile range was considered more representative than the mean ± 2SD, which is susceptible to extreme values.

In clinical contexts, pathological stomatocytosis primarily includes hereditary stomatocytosis (HSt) a spectrum of hemolytic anemias due to increased cation permeability of the erythrocyte membrane, which is typically associated with hemolysis, anemia, and abnormal laboratory findings (Andolfo et al. 2025; Flatt and Bruce 2009). In addition to hereditary forms, “acquired” stomatocytes can occur in liver disease/alcohol use disorder, certain cationic drugs, and lipid metabolism disorders (Wislöff and Boman 1979; Zini et al. 2021). The absence of liver/renal function abnormalities and hematologic parameters in this study supports the interpretation that the low stomatocyte frequency does not reflect an active pathological process.

Variation by Gender

The slightly higher median stomatocyte count observed in men (4/1000 erythrocytes) compared with women (3/1000 erythrocytes) may reflect sex-related physiological differences in erythropoiesis and red blood cell turnover, which are also known to influence hemoglobin concentration and hematocrit values (Bachman et al. 2014; Murphy 2014). Androgen

hormones, particularly testosterone, stimulate erythropoietin production and enhance erythroid progenitor proliferation, leading to increased red blood cell mass and potentially greater variability in erythrocyte morphology. Estrogen has been shown to stabilize erythrocyte membrane composition and oxidative balance, which may contribute to reduced morphological variation in women. Furthermore, sex-specific differences in erythrocyte membrane lipid composition, intracellular ion transport, and cell hydration status may affect the propensity for transient, nonpathologic stomatocytic shape changes. These subtle morphological variations are generally regarded as physiological and do not indicate underlying hematological disease when present at low frequencies. However, due to the limited sample size and the predominance of male participants in the present study, the observed difference may reflect sampling variability rather than a true biological effect. Therefore, this finding should be interpreted cautiously and requires validation in future studies with larger, sex-balanced cohorts and controlled assessment of confounding variables such as age, hydration status, and pre-analytical factors.

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Reference Range of Stomatocytes in Jakarta and Surrounding Areas

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Abstract

Stomatocytes are erythrocytes distinguished by a slit-like central pallor. While they may occur in small numbers under physiological conditions, elevated proportions are linked to pathological states. Establishing population-specific reference intervals is crucial to prevent misinterpretation of erythrocyte morphology. This study determined the reference interval for stomatocyte counts in a healthy population from Jakarta and surrounding areas and evaluated their association 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 assessed from peripheral blood smears by counting per 1,000 erythrocytes. Reference intervals were calculated using the 2.5th to 97.5th percentile method. The reference interval for stomatocytes in the overall population was 0 to 1.85%. Sex-specific reference intervals were 0 to 2.0% in men and 0 to 1.7% in women. No significant associations were found between stomatocyte counts and complete blood count parameters, liver function tests, or kidney function tests. In summary, stomatocytes may be present at low levels in healthy individuals, with a locally derived reference interval of 0 to 1.85%. These results underscore the necessity of applying clearly defined reference intervals and considering clinical context when interpreting erythrocyte morphology to avoid overdiagnosis.

Keyword: Stomatocytes, reference values, abnormal erythrocytes, peripheral blood smear.

INTRODUCTION

Erythrocytes transport oxygen and carbon dioxide throughout the body's tissues. Their biconcave structure confers high flexibility, enabling passage through narrow vascular spaces and providing a large surface area for gas exchange. This structural feature, however, also renders erythrocytes vulnerable to morphological changes under pathological or physiological stress. Stomatocytes represent one such abnormality, characterized by an elliptical or lip-shaped central pallor arising from disruptions 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 essential

because the presence of stomatocytes may indicate underlying membrane instability or systemic disorders and carries significant implications for hematological assessment.

The morphological variation of red blood cells, including stomatocytes, necessitates standardization of terminology, recognition criteria, and reporting methods to ensure interlaboratory consistency. The International Council for Standardization in Hematology (ICSH) has published guidelines for the nomenclature and grading of morphological features of peripheral blood cells, as well as updated recommendations for the quantitation of some fragment forms (e.g., schistocytes) to prevent diagnostic misinterpretation (Melzak et al. 2018; Palmer et al. 2015; Zini et al. 2021). While the ICSH's focus on schistocyte thresholds does not directly establish stomatocyte thresholds, the spirit of standardizing morphology on blood smears provides a framework for local reporting practices and validation of low-number abnormal cell findings (Andolfo, Iolascon, and Russo 2025; Palmer et al. 2015; Zini et al. 2021).

Pathobiologically, stomatocytes can reflect impaired erythrocyte membrane permeability and cation homeostasis (Na^+/K^+) in hereditary conditions such as dehydrated hereditary stomatocytosis/hereditary xerocytosis (Andolfo et al. 2025), as well as changes in membrane lipid composition in alcohol-related liver disease, which can cause stomatocyte shapes and resolve with improvement in liver function (Ridwan, Agustina, and Makmur 2021). However, in healthy individuals, cells with stomatocyte-like morphology can also occasionally appear due to drying artifact, which is why assessment should encompass multiple smear areas and not rely on a single field of view (Chen et al. 2023). This reinforces the urgency of having local reference values for stomatocyte counts so that clinicians can differentiate physiological variations from disease manifestations.

Some references state that healthy individuals have a stomatocyte prevalence of less than 3%. In comparison, an increase in the number exceeding 5% is correlated with pathological conditions such as hereditary stomatocytosis, liver cirrhosis, fatty liver, and hemolytic anemia (Körber et al. 2017; Pozdnyakova 2025; Romanenko et al. 2024). To date, Indonesia lacks a standard reference for stomatocytes, and we still rely on data from populations abroad, such as those in America and Europe. This is because there is limited research on this topic in Indonesia. Determining normal values in Indonesia is very necessary due to the influence of genetic, ethnic, and environmental factors.

Based on this, we need to establish local reference values to improve diagnostic accuracy related to changes in erythrocyte shape in Indonesia. Therefore, this study aims to determine

the reference values for stomatocytes in a healthy adult population in Jakarta and its surrounding areas, and to evaluate potential differences in these values based on gender.

METHODS

A descriptive cross-sectional design was utilized, with primary data collected through laboratory examinations and peripheral blood smear analyses. The research 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 timeframe, 65 adult patients undergoing routine medical check-ups were initially identified and screened for eligibility. Of these, 54 samples were included in the final analysis following the application of predefined inclusion and exclusion criteria. Eleven samples were excluded due to patient age outside the targeted range of 21 to 60 years or laboratory test results that did not meet the required normal reference values, which could confound erythrocyte morphology assessment. The target population comprised adult patients 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 employed, including all subjects who met the eligibility criteria during the study period to minimize selection bias and ensure comprehensive data representation. Inclusion criteria required patients 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 encompassed incomplete medical records, pregnancy or breastfeeding, a history of 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 included EDTA tubes, slides, dropper pipettes, staining racks, light microscopes, and staining equipment (methanol, Wright stain, distilled water, immersion oil). The biological material was a venous blood sample from each subject. If the sample met the inclusion criteria, the study began with the preparation of a peripheral blood smear with one drop of EDTA blood taken from the vein, placed on a slide, and then the blood smear was made and fixed using methanol and Wright stain. The preparation was observed under a light

microscope at 1000x magnification using immersion oil (Chen and Boyle 2017). Recording was carried out using a microscope camera. After reaching 1,000 erythrocytes, a search was conducted for stomatocytes. The researcher recorded the number of stomatocytes found. Furthermore, the examination results were verified by one clinical pathologist to ensure accuracy (Ridwan et al. 2021). Data were analyzed univariately by calculating the median and the 2.5th to 97.5th percentile range as reference values, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. Normality tests and data distribution analysis were conducted using SPSS software version 27. If the data were not normally distributed, the median was used as a measure of central tendency and the percentile range as a measure of distribution (Liana et al. 2022; Rosida and Hendriyono 2015).

RESULTS

This study involved 54 subjects, consisting of 37 men (68.52%) and 17 women (31.48%). All subjects were healthy individuals, as determined by medical check-ups and laboratory results, which included basic hematology and biochemistry parameters, and met the established inclusion and exclusion criteria. Subjects were distributed in the age range of 21–60 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 Sex

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 among all examined peripheral blood smears. The percentage range of 0–1.85% indicates that, in most samples, stomatocytes were not detected, and when present, they appeared only sporadically and did not exceed the upper threshold commonly considered within normal morphological variation. When stratified by sex, male subjects showed a slightly higher maximum percentage (0–2.00%) compared to female subjects (0–1.70%); however, this difference is minimal and not clinically significant. The absence of prominent stomatocytosis in the majority of samples is consistent with the strict inclusion criteria applied in this study, which selected individuals with normal hematological and biochemical parameters and no history of conditions known to affect erythrocyte membrane stability. Therefore, these findings suggest that stomatocytes observed in this population likely represent normal morphological variants rather than pathological abnormalities.

DISCUSSION

The presence of stomatocytes at low percentages in healthy individuals suggests that this erythrocyte morphology can represent a physiological variation rather than an inherent pathological condition. Stomatocytes are characterized 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, fall well within the limits reported in previous literature, which describe stomatocytes occurring in up

to 3% of erythrocytes in healthy populations (Perrin et al. 2015; Pozdnyakova 2025). The observed differences between sexes may be attributable to 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 causing clinically significant hematological abnormalities. Importantly, the low prevalence of stomatocytes observed in this study does not indicate an increased risk of future hematological disorders in the participants, as isolated stomatocytosis at minimal levels is not associated with hemolysis, anemia, or functional impairment of red blood cells (Fusi et al. 2024; Geekiyanage et al. 2020). Pathological significance 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 are formed due to disturbances in osmotic balance and cell membrane structure, including genetic mutations (PIEZO1, KCNN4, RHAG) or exposure to certain drugs. However, there were no clinical or biochemical indications pointing to a pathological etiology in this study population (Achfidawati et al. 2019; Flatt and Bruce 2009). The 2.5–97.5% percentile method was chosen because the distribution of stomatocyte data was not normal. The use of the median and percentile range was considered more representative than the mean ± 2SD, which is susceptible to extreme values.

In clinical contexts, pathological stomatocytosis primarily includes hereditary stomatocytosis (HSt) a spectrum of hemolytic anemias due to increased cation permeability of the erythrocyte membrane, which is typically associated with hemolysis, anemia, and abnormal laboratory findings (Andolfo et al. 2025; Flatt and Bruce 2009). In addition to hereditary forms, “acquired” stomatocytes can occur in liver disease/alcohol use disorder, certain cationic drugs, and lipid metabolism disorders (Wislöff and Boman 1979; Zini et al. 2021). The absence of liver/renal function abnormalities and hematologic parameters in this study supports the interpretation that the low stomatocyte frequency 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 sex-related physiological differences in erythropoiesis and red blood cell turnover, which are also known to influence hemoglobin

concentration and hematocrit values (Bachman et al. 2014; Murphy 2014). Androgen hormones, particularly testosterone, stimulate erythropoietin production and enhance erythroid progenitor proliferation, leading to increased red blood cell mass and potentially greater variability in erythrocyte morphology. Estrogen has been shown to stabilize erythrocyte membrane composition and oxidative balance, which may contribute to reduced morphological variation in women. Furthermore, sex-specific differences in erythrocyte membrane lipid composition, intracellular ion transport, and cell hydration status may affect the propensity for transient, nonpathologic stomatocytic shape changes. These subtle morphological variations are generally regarded as physiological and do not indicate underlying hematological disease when present at low frequencies . However, due to the limited sample size and the predominance of male participants in the present study, the observed difference may reflect sampling variability rather than a true biological effect. Therefore, this finding should be interpreted cautiously and requires validation in future studies with larger, sex-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 due to physiological variation or pre-analytical and analytical artifacts, rather than to underlying pathology. Variables such as smear preparation technique, drying time, anticoagulant effects, and field selection during microscopic evaluation can affect erythrocyte morphology and lead to the incidental observation of stomatocytes. Consequently, evaluation across multiple microscopic fields and, when indicated, repeat smear examination are necessary 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, especially at low frequencies, is insufficient for the diagnosis of hereditary stomatocytosis. Hereditary stomatocytosis is a rare hemolytic disorder defined 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 transportmechanisms. 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 sex-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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