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NORMAL BURR CELL VALUE RANGE IN HEALTHY ADULTS

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ABSTRACT
Burr cells or also known as echinocytes are erythrocytes with short spicules evenly spaced and appear pale in the middle. Burr cells are found in patients with chronic kidney disease, burns, liver disease, uremia, microangiopathic hemolytic anemia, pyruvate kinase deficiency, post-transfusion, or can be found in the form of artifacts. This study aims to find the range of normal burr cell values in healthy adults and to find the correlation between burr cells with urea, creatinine, cholesterol, SGPT, and gender. The method used is a quantitative descriptive research design with a cross-sectional approach. Samples were taken using a purposive sampling technique, and 54 subjects who met the inclusion and exclusion criteria were analyzed for patient characteristics and laboratory results. The results showed a very weak relationship between burr cells with gender, creatinine, and SGPT with a correlation value of <0.1 and a weak relationship between burr cells with urea and cholesterol with a correlation value of >0.1 - <0.3. The normal burr cell value range was obtained with a lower limit value of 0 and an upper limit value of 30.88 burr cells per 1000 erythrocytes.

Keywords: burr cell; cholesterol; creatinine; urea

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INTRODUCTION

Erythrocytes are one of the blood components that play a role in carrying and distributing oxygen to all body tissues. Erythrocytes are biconcave disc-shaped, have no nucleus, and do not move. The normal reference for erythrocytes ranges from 4.6 - 6.2 million cells / mm3 in adult men and 4.2 - 5.4 million cells / mm3 in adult women (Brihi, 2024). The quality and color of blood are determined by hemoglobin levels. Hemoglobin is a molecule consisting of four hemes and four globin chains (alpha, beta, gamma, delta). Heme itself is a non-protein component with Fe2+ content. This iron ion acts as an oxygen binding site. Normally, hemoglobin levels in the blood range from 13 - 18 g / dL in men and 12 - 16 g / dL in women (Aridya & Yuniarti, 2023). Erythrocytes are essential cells with the largest number in the human body, ranging from 20 - 30 trillion and covering almost 70% of the total number of cells in the average adult. The formation of erythrocytes is called erythropoiesis, starting with the differentiation of multipotent hematopoietic stem cells in the bone marrow. Hematopoietic Stem Cells will differentiate into myeloid progenitors, which will later become cells called erythroblasts. Erythroblasts develop through the polychromatic stage to the orthochromatic stage of erythroblasts (Abramson, 2006). At this stage, the erythroblast nucleus will become increasingly condensed (dense) in preparation for enucleation. The nucleus released from the erythroblast cell will be digested by macrophages.

These cells without a nucleus are then called reticulocytes, released into the blood circulation, and maturing into erythrocytes within 1 - 2 days (Rosida & Hendriyono, 2015). During the process of maturation of reticulocytes into erythrocytes, the remaining organelles in the reticulocytes will be destroyed. Enucleation and the process of destroying the remaining organelles allow the red blood cells to have more space to transport hemoglobin. Disruption of the enucleation process can lead to the production of abnormal red blood cells (Anderson et al., 2018; Dzierzak & Philipsen, 2013). The normal life span of erythrocytes is 120 days. If the erythrocyte membrane is deformable, the structure and function of hemoglobin are adequate, and the osmotic balance and permeability of erythrocytes are maintained (Situmorang et al., 2023). Erythrocytes require ATP; decreased ATP levels will cause: damaged membrane lipids, accumulation of intracellular Na+ and Ca2+, decreased K+ and intracellular H2O levels, cell dehydration, which can cause the membrane to be stiff and easily broken. Burr cells are abnormalities in the shape of erythrocytes due to dehydration. Decreased deformability is also considered related to decreased ATP levels, which will result in impaired erythrocyte function to transport sugar (Wismaya, 2019). Burr cells or also known as echinocytes are erythrocytes with short spicules evenly spaced and appear pale in the middle. Burr cells are found in chronic kidney patients, the presence of lipid membrane peroxidation by free radicals will cause membrane changes.

Burr cells are associated with azotemia. In addition, the appearance of burr cells is also influenced by sample storage time, plasma osmolarity, EDTA exposure, high pH, temperature, and dialysis (Ahmed & Patel, 2015; Maharani & Noviar, 2018). Diseases and clinical status related to burr cells include: artifact, post-transfusion, burns, liver disease, uremia, microangiopathic hemolytic anemia, pyruvate kinase deficiency (Villatoro & To, 2019). Burr cells are seen in a rare hereditary disorder, abetalipoproteinemia. It is also a feature of hepatic coma, where a large number of these cells is considered a sign of poor prognosis (Hoffbrand et al., 2019). There is no valid reference value for burr cells. A study conducted by Ahmed M. and Patel A. R. analyzed 148 peripheral smears of healthy adults to detect the normal range of burr cell values. In the study, burr cells were observed in 114 normal adults, with an average burr cell count of 0.051% (Pilichiewicz et al., 2009). As a conclusion from the explanation above, burr cells are an abnormality of erythrocyte shape that needs to be considered. To support the accuracy of the diagnosis, there needs to be a valid reference value for burr cells that has been tested by many researchers. Therefore, the researchers feel that this study is very necessary to find the reference value of burr cells. This study aimed to determine the normal range of burr cell values in healthy adults and their correlation with urea, creatinine, cholesterol, SPGT, and gender.

METHOD

Research Design

This study uses a quantitative descriptive research method with a cross-sectional approach. Quantitative descriptive research is describing, examining, explaining, and drawing conclusions from observed phenomena using numbers (Nurhabiba & Misdalina, 2023). The cross-sectional approach is used to study the dynamics of the correlation between risk factors and effects by observing and collecting data from a population, and is used only once. In this case, the researcher studied a phenomenon of erythrocyte deformity, namely burr cells, and sought a correlation between the number of burr cells per 1000 erythrocytes with the parameters studied, namely urea, creatinine, cholesterol, SPGT and gender (Abduh et al., 2023).

Place and Time of Research

The research was conducted from January 2022 to December 2023 at one of the Private General Hospitals in East Bekasi and at the General Hospital of the Christian University of Indonesia.

Population and Sample

The population in this study were all healthy adult patients who underwent a Medical Check Up at one of the Private General Hospitals in East Bekasi and at the Indonesian Christian University Hospital; sampling based on the Guidelines from the Clinical and Laboratory Standards Institute, the best way to find the normal burr cell value range is to collect at least 120 samples that meet the research requirements, in a non-parametric way. Alternatively, researchers can validate the existing normal or reference value range by collecting at least 20 samples. Samples were taken using a purposive sampling technique, and 54 subjects who met the inclusion and exclusion criteria were analyzed for patient characteristics and laboratory results. So that researchers can conduct a simple binomial test and can validate the normal burr cell value range in their population and methodology (Horowitz et al., 2010).

Data Analysis and Processing

The data collected are in the form of numbers that describe the frequency of burr cells per 1000 erythrocytes in each sample. Then, the data is checked to ensure that there are no errors in the calculations or that there are blood samples that do not meet the analysis criteria. After the data is collected, further data processing will be carried out using the application to obtain descriptive statistics (eg, mean, median, and data distribution). Given the limited number of researcher samples, which is 54 samples, and the possibility of non-normal data distribution, the researcher will use a non-parametric analysis method. Based on guidelines from the Clinical and Laboratory Standards Institute, the non-parametric approach that is suitable for data with a small number of samples is to use percentiles.

Determining Reference Values

Percentiles in this non-parametric method indicate the lower and upper limits for the number of burr cells that are considered normal. Here are the steps to determine the range of normal values using percentiles:

1. Sorting data: by sorting the number of burr cells per 1000 erythrocytes from the lowest to the highest value.
2. Determining percentiles: where the 2.5th percentile will be the lower limit and the 97.5th percentile will be the upper limit. This will provide a range of normal values that includes 95% of normal samples.

RESULT

Sample Characteristics

In this study, there were 54 samples that met the inclusion criteria, with an age range of 18 – 59 years.

Table 1.
Characteristic of Participant based on Age

	N	Minimum	Maximum	Mean	Std. Deviation
Age	54	18	59	38,59	11,619

Based on the data, the age range of the subjects studied was 18-59 years. The average age of the subjects studied was 38.59 years, and had a standard deviation value of 11.619. SD shows that there are many individuals whose ages are far above or below the average (approaching the minimum value of 18 years or the maximum value of 59 years). Most of the age data is in the age range of 26.97-50.21 years.

Table 2.
Sample Characteristics Based on Gender

Gender			Statistic	Std. Error
Number of Burr Cells per 1000 erythrocytes	Male	Mean	2.22	.628
		Median	.95	
		SD	3.765	
		Minimum	0	
		Maximum	16	
		Skewness	2.044	.393
	Female	Mean	4.50	2.311
		Median	.00	
		SD	9.805	
		Minimum	0	
		Maximum	38	
		Skewness	2.808	.536

Based on the data, men have a lower average number of burr cells than women (2.22 burr cells per 1000 erythrocytes). The median of 0.95 indicates that half of the adult male samples have a burr cell count ≤ 0.95 per 1000 erythrocytes. The data range of 0-16 shows a smaller variation in the number of burr cells compared to women. In data group 2 (women) the average number of burr cells is 5.18 per 1000 erythrocytes. The data range of 0-38 shows a greater variation in the number of burr cells compared to men. Skewness 2.808 shows more high extreme values found in women.

Bivariate Analysis

Bivariate analysis is used to see the relationship between independent variables and dependent variables. Independent variables in this study include: age, gender, urea, creatinine, cholesterol, and SPGT levels. The dependent variable in this study is the number of burr cells per 1000 erythrocytes and sample data analysis by determining the range of normal burr cell values.All bivariate analyses were performed using the SPSS application. The statistical test used in this study is the Spearman correlation test. This correlation test is more suitable because the existing data is ordinal or not normally distributed, to measure the strength of the monotonic relationship.

Table 3.
Distribution of Burr Cell Count in Male and Female Patients

Normality Test					
		Kolmogorov-Smirnov		Shapiro-Wilk	
	Gender	Statistic	Sig.	Statistic	Sig.
Number of Burr Cells per 1000 Erythrocytes	Male	.294	.000	.661	.000
	Female	.339	.000	.544	.000

Based on the data, in both tests, Kolmogorov-Smirnov and Shapiro-Wilk showed a Sig. value = 0.000 or < 0.05. This indicates that the data on the number of burr cells in men and women are not normally distributed. Therefore, parametric statistical tests cannot be used.

Table 4.
Spearman Correlation Test between Number of Burr Cells per 1000 Erythrocytes and Gender

			BC Male	BC Female
Spearman's rho	BC <ale	Correlation Coef.	1.000	.080
		Sig. (2-tailed)		.751
		N	36	18
	BC Female	Correlation Coef.	.080	1.000
		Sig. (2-tailed)	.751	
		N	18	18

Table 5.
Spearman Correlation Test between Burr Cell Count and Urea

			BC count / 1000 erythrocytes	Urea
Spearman's rho	BC count / 1000 erythrocytes	Correlation Coef.	1.000	.106
		Sig. (2-tailed)		.445
		N	54	54
	Urea	Correlation Coef.	.106	1.000
		Sig. (2-tailed)	.445	
		N	54	54

Table 6.
Spearman Correlation Test between Burr Cell Count and Creatinine.

			BC count / 1000 erythrocytes	Creatinine
Spearman's rho	BC count / 1000 erythrocytes	Correlation Coef.	1.000	.094
		Sig. (2-tailed)		.500
		N	54	54
	Creatinine	Correlation Coef.	.094	1.000
		Sig. (2-tailed)	.500	
		N	54	54

Table 7.
Spearman Correlation Test between Burr Cell Count and Cholesterol.

			BC count / 1000 erythrocytes	Cholesterol
Spearman's rho	BC count / 1000 erythrocytes	Correlation Coef.	1.000	.202
		Sig. (2-tailed)		.143
		N	54	54
	Cholesterol	Correlation Coef.	.202	1.000
		Sig. (2-tailed)	.143	
		N	54	54

Table 8.
Spearman Correlation Test between Burr Cell Count and SPGT

			BC count / 1000 erythrocytes	SPGT
Spearman's rho	BC count / 1000 erythrocytes	Correlation Coef.	1.000	.071
		Sig. (2-tailed)		.612
		N	54	54
	SPGT	Correlation Coef.	.071	1.000
		Sig. (2-tailed)	.612	
		N	54	54

Table 9.
Normal Burr Cell Value Range

Number of Burr Cells per 1000 erythrocytes		
N	Valid	54
	Missing	0
Mean		2.98
Std. Deviation		6.432
Minimum		0
Maximum		38
Percentiles	2.5	.00
	97.5	30.88

DISCUSSION

Correlation between Burr Cell Count and Gender

Based on the data, the correlation value of 0.080 indicates a very weak relationship between burr cell count and gender. A positive sign indicates a tendency for burr cell count to be higher in women than in men, but the strength is so small that there is almost no relationship. Based on the theory, women do tend to experience decreased hemoglobin levels, which affect the shape and size of erythrocytes. This is associated with the large amount of blood lost during menstruation, which forces the use of iron reserves in women (Septiyani Putri, 2021)

Correlation between Burr Cell Count and Urea

Based on the data, the correlation value of 0.106 indicates a weak relationship between the number of burr cells and urea levels. A positive relationship indicates that if urea levels increase, the number of burr cells will also increase, but this relationship is not very significant. Uremia is a clinical syndrome that occurs due to decreased kidney function, characterized by increased levels of urea and creatinine in the blood (Rasyid, 2021). One diagnosis of uremia is to make a peripheral blood smear and look for burr cells. There are several mechanisms by which high urea levels can contribute to the formation of burr cells:

1. Metabolic acidosis: When the body accumulates acid or loses base, the blood pH will decrease. The erythrocyte membrane is very sensitive to decreased pH. Decreased pH can change the structure of lipids and proteins in the membrane which leads to the formation of burr cells.(Kandrashina et al., 2024)
2. Increased oxidative stress: Free radicals can damage the membrane and cause deformation of the cell shape.
3. Disruption of ion transport: in acidosis conditions, especially sodium and potassium ions. Ion imbalance will cause the formation of burr cells.
4. Changes in blood viscosity: increased blood viscosity causes blood flow to slow down, this also contributes to the formation of burr cells (Kandrashina et al., 2024; Mahaputra & Putra, 2018)

Correlation between Burr Cell Count and Creatinine

Based on the data, the correlation value of 0.094 indicates a very weak and positive relationship between the number of burr cells and creatinine levels. One of the mechanisms that connects creatinine with burr cells is the accumulation of metabolic waste products (uremia), uremia causes ion and osmotic imbalances that disrupt membrane stability. While creatinine does not directly cause the formation of burr cells, but increased creatinine levels indicate impaired kidney function that can cause burr cells (Barbalato & Pillarisetty, 2025)

Correlation between Burr Cell Count and Cholesterol

Based on the data, the correlation value of 0.202 indicates a weak and positive relationship between the number of burr cells and cholesterol levels. Cholesterol is an integral component of the membrane. Increased cholesterol in the blood can cause an increase in cholesterol content in the erythrocyte membrane, excessive accumulation can change the distribution of lipids in the membrane and cause an imbalance between the inner and outer layers of the lipid bilayer membrane. Changed membrane lipid composition can also affect interactions with membrane proteins (actin and spectrin) that play a role in maintaining the shape of erythrocytes (Barbalato & Pillarisetty, 2025; Mallah et al., 2010).

Correlation between Burr Cell Count and SGPT

Based on the data, the correlation value of 0.071 indicates a very weak relationship between the number of burr cells and SGPT levels. SGPT is an enzyme used as an indicator to assess liver damage. Several mechanisms can explain the relationship between SGPT and burr cells:
1. Liver damage disrupts lipid metabolism.
2. In some liver disorders (eg: cirrhosis), there is an increase in the formation of abnormal erythrocytes, including burr cells (NURPALAH, 2018).

Normal Burr Cell Value Range

This study used 54 blood samples from healthy adults aged 18 to 59 years. The number of burr cells was counted manually in 1000 erythrocytes in each sample. The data obtained were then analyzed to determine the mean (± 2SD) as a method of finding the normal value range if the data distribution is normal. If not normally distributed, the 2.5th and 97.5th percentiles are used. From the results of the erythrocyte calculation, the number of erythrocytes in men was: 0, 4, 0, 0, 8, 1, 0, 0, 0, 0, 1, 0, 0, 0, 0, 2, 0, 0, 3, 10, 0, 1, 7, 0, 2, 0, 0, 0, 2, 8, 16, 8, 1, 6, 0, 0.
In women, the number of erythrocytes was: 2, 0, 0, 0, 0, 6, 0, 0, 0, 0, 1, 19, 38, 12, 0, 3, 0, 0.
So that the average of the total of male and female subjects was obtained: 2.98. Together with the SD obtained from the square root of 41.377 (variance), 6.432 was obtained as the SD.
Then the normal value range is obtained:
 Lower limit value: mean – 2SD = 2.98 – (2 x 6.432) = 0.
 Upper limit value: mean + 2SD = 2.98 + (2 x 6.432) = 15.844 = 0.0158 or 1.58%.
Because the data distribution is not normal, with a P value <0.05. So to determine the normal value it would be better to use percentiles, because the mean as the center of the data will be affected by outliers or skewed distributions. Percentiles do not require distribution assumptions; this method only calculates data in order, covering 95% of the data in the middle so that it is more robust to outliers.

In this study, the percentile value for the lower limit was 0, and the lower limit was 30.88. There are several possibilities why the normal range of burr cell values studied was high. One of the main possibilities is the presence of crenation cells. Burr cells and crenation cells are similar in shape even with similar causative factors (eg: due to electrolyte imbalance, dehydration, kidney disorders, anemia). The phenomenon of crenation cells can also arise due to errors in the staining process:
1. Fixation or dye that is too long or too strong.
2. Dehydration in the staining process: due to suboptimal drying.
3. Uneven staining (Linda, 2021).
Then, the researcher tried to remove samples with extreme values 38, 19, 16, and 12 from the database and recalculated using SPSS. The lower limit value was 0, and the upper limit value was 9.45. With an average burr cell of 0.152%.

CONCLUSION

Based on the research conducted, the normal range of burr cell values in healthy adults is 0 to 1.58% (mean ± 2SD) or 0 to 30.88 burr cells per 1000 erythrocytes (with percentile). This normal range can be used as a temporary benchmark for burr cell diagnosis although it still requires further validation in a wider population. A very weak and positive relationship was found between the number of burr cells and gender, creatinine levels, and SGPT with a correlation value of <0.1. There is a weak and positive relationship between the number of burr cells and urea and cholesterol levels with a correlation value of> 0.1

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