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Analysis of Circadian Rhytme Variations on Glucose Examination Results

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Abstract: Background: Circadian rhythm is a term used to describe the 24-hour physiological rhythm of organisms. According to Minister of Health Regulation no. 43 of 2013 concerning How to Organize a Good Clinical Laboratory, the Circadian rhythm variation is explained in the body as levels of certain substances change from time to time. Changes in substance levels that are influenced by time can be cyclical, such as daily cycles (diurnal variations), monthly cycles (menstruation), or linear/straight lines, such as age. Classic experiments using a 72-hour glucose clamp methodology, demonstrated that humans exhibit a robust Circadian rhythm in insulin secretion, independent of food and glycemia. Additionally, a cell-autonomous Circadian clock appears to oversee the diurnal rhythm in glucose-induced insulin secretion.Research Objectives: Knowing the analysis of Circadian rhythm variations on glucose examination results. Research Methods: Analytical observational analysis using cross sectional methods and purposive sampling techniques. The sample population of applied TLM undergraduate students class of 2020 had blood serum taken and using the Slovin formula to determine the sample size, 36 samples were obtained with respondents without a history of diabetes mellitus. Data analysis used the Paired t-test statistical test. Research Result: The results obtained from the examination of the average glucose level at the time of sampling in the morning were 80.25 mg/dl, in the afternoon 96.5 mg/dl, and in the evening 85.91 mg/dl. The results of the paired t-test statistical analysis obtained Sig. (2-tailed) or p 0.010 means $p < 0.05$ there is a difference in the average glucose results at the time of sampling in the morning and afternoon and Sig. (2-tailed) or p 0.115 means $p > 0.05$ there is no difference in the average glucose test results at the time of sampling in the morning and evening. Conclusion: Blood glucose levels taken in the morning have lower average levels than during the day and evening.

Keywords: Variation; Circadian rhythm; Glucose

Introduction

A health laboratory is a place where samples of human or non-human origin are measured, examined and identified for diseases, conditions or variables that affect health. Clinical laboratories can provide examination services to promote diagnosis and health recovery in the medical field (Annisa Nur Aini et al., 2022; Apriani et al., 2023). The pre-analytic, analytical and post-analytic stages are three important stages in the clinical laboratory examination process (Bastian & Ulva, 2023; Kusmiati et al., 2022; Sun, 2022). In quality control supervision, the inspection stage that is often prioritized only concentrates on analytical and post-analytical, despite the fact that the pre-analytical stage is given less attention. In fact, errors in the pre-analytical stage affect the examination results by 61% compared to 25% in the analytical stage and 14% in the post-analytical stage (Nabilah et al., 2023). Patient preparation is the most important pre-analytic stage in carrying out an examination (Amani et al., 2019; Anggraini et al., 2022).

Based on this statement, the author will focus on discussing the pre-analytical laboratory stage in patient preparation which can have an impact on the results of examinations in the laboratory (Bonham et al., 2019; Cheng et al., 2024). Patient preparation refers to a series

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of actions and preparations that must be carried out before the patient undergoes tests or laboratory examinations. According to Minister of Health Regulation no. 43 of 2013 How to Organize a Good Clinical Laboratory, that at the patient preparation stage, there are several factors that can influence the results of laboratory examinations. These factors include diet, drug consumption, smoking, alcohol use, physical activity, altitude, fever, trauma, age, race, gender and pregnancy (Ayu Yolanda et al., 2019; Fanana Mahdia et al., 2018; Larsen et al., 2020). Apart from that, there are also other factors in patients that can have an impact on examination results, namely variations in *Circadian rhythm* (Lee et al., 2021; Li et al., 2021).

The term *Circadian rhythm* is used to describe the body's physiological rhythms throughout the day. In the bodies of living creatures there are fluctuations or changes in temperature, the ability to wake up, blood pressure, hormone levels, and heart rate which change during 24 hours(Fatima & Rana, 2020). In general, in the morning body functions will increase and begin to weaken during the day and continue to decline at night for recovery and renewal (Maulana et al., 2014). According to Minister of Health Regulation no. 43 of 2013, in the *Circadian rhythm variations* explained in the body the levels of certain substances change from time to time. These changes are influenced by cyclical time, such as daily cycles (diurnal variations), monthly cycles (menstruation), or linear/straight (Keramat et al., 2023; Kramer et al., 2022; Mamalaki et al., 2019)

lines, such as age. In this research, the author will examine *Circadian rhythm variations* in a person's daily or diurnal cycle. In the diurnal *Circadian rhythm variations* that occur indicate glucose, namely in the morning, insulin levels will reach their peak (Sun, 2022). It is also associated with fluctuating blood glucose levels, which can be influenced by various factors (Wijayanti et al., 2020). Classic experiments using a 72-hour glucose clamp methodology, demonstrated that humans exhibit a robust circadian rhythm in insulin secretion, independent of food and glycemia. Moreover, a cell-autonomous circadian clock appears to supervise the diurnal rhythm in glucose-induced insulin secretion (Hariri et al., 2023). Based on this background, the author wants to analyze the diurnal or daily variations of *Circadian rhythm* on the results of blood glucose examinations in the morning, afternoon and evening and it is hoped that this research can be useful as clinical input for medical laboratory techniques staff.

Method

This study aims to analyze Circadian rhythm variations in glucose examination results using blood serum samples to find the best time to take samples at the pre-analytical stage. The research was carried out at the Dasan Tapen Health Center Laboratory, West Lombok. The sample population, namely applied TLM undergraduate students from the class of 2020, had blood serum taken and using the *Slovin formula* to determine the sample size, 36 samples were obtained. Samples were taken from 12 respondents and blood was drawn from each respondent three times, namely in the morning, afternoon and evening. The morning time was given special treatment, namely sampling before eating, while the afternoon and evening times were controlled for eating 2 hours or more after eating. Blood serum samples were examined using a spectrophotometer with a wavelength of 546 λ (Nuzhatul Sabrina Farikha et al., 2023).

This research uses an observational analytical cross-sectional approach and the sampling technique is purposive with the criteria that the respondent does not have a history of diabetes mellitus. In this study there are two types of variables, namely the independent variableiin i research i is a variation of Circadian *rhythm and* variable i dependent *is* glucose test results (Lee & Wisor, 2022; Todd et al., 2020). Data from research on analysis of circadian rhythm variations on glucose examination results were analyzed using the *Paired sample t-test statistical test* using SPSS (95% confidence level probability < α = 0.05) which was used to draw conclusions.

Result and Discussion

Table 1 Shows the results of research from 36 specimens, the average glucose level in the morning was 80.25 mg/dl, during the day it rose to 96.5 mg/dl, and at night it fell to 85.91 mg/dl. etc. Based on the data, it shows that the average glucose test results during the day are higher than in the morning and evening but are still classified as normal glucose levels, namely <160 mg/dl. The average glucose level in the morning has the lowest value compared to the afternoon and evening. Data analysis uses the Paired t- test with the condition that a normality test is carried out first and the data is normally distributed. The Shapiro-Wilks normality test method was used. From the data above, it shows that the morning sample results are sig. 0.878, midday sample sig. 0.511, night sample sig. 0.311, all have a probability value > 0.05, the data is normally distributed.

The result of the Paired t-test is the Sig value. (2-tailed) pair 1 namely 0.010 < 0.05, then Ho is rejected and HA is accepted, there is a difference in the average glucose test results when sampling in the morning and afternoon. Sig output value. (2-tailed) pair 2, namely 0.115 > 0.05 and Sig. (2-tailed) pair 3, namely 0.131 > 0.05, then Ho is accepted and HA is rejected, there is no

difference in the average glucose test results for morning and evening sampling times, as well as afternoon and evening sampling times.

Table 1 Results of research examining glucose levels in the morning, afternoon and evening

No.	Sample Code (Respondent)	Serum Glucose Examination Results		
		Morning Sample (mg/dl)	Sampel Siang (mg/dl)	Sampel Malam (mg/dl)
1	A1	80	101	96
2	A2	83	86	90
3	A3	91	127	75
4	A4	87	99	76
5	A5	77	98	96
6	A6	75	89	97
7	A7	78	69	86
8	A8	84	115	81
9	A9	75	72	83
10	A10	78	134	90
11	A11	72	83	77
12	A12	83	85	84
Jumlah		963	1158	1031
Rata-rata		80,25	96.5	85.91

Note: morning samples=taken at 07.00 – 08.00 WITA, afternoon samples=taken at 13.00 – 14.00 WITA, evening samples=taken at 17.00 – 18.00 WITA

Circadian rhythm is the body's intrinsic rhythm regulated by the hypothalamus which acts as the human body's natural clock. This circadian rhythm is a time recognition rhythm that is adjusted to the rotation of the Earth during a 24 hour cycle. This rhythm is shared by almost all living creatures, which consistently experience changes in physical and bodily functions during a 24-hour cycle (Ambarwati et al., 2017; Raymundo & Reis, 2018). During 24 hours, body temperature, ability to wake up, blood pressure, stomach activity, heart rate and hormone levels will fluctuate (Ambarwati et al., 2017) Blood glucose is a simple sugar in the form of disaccharides found in food. Blood glucose levels refer to the level of glucose in the blood whose concentration is tightly regulated in the body. There are endogenous and exogenous factors that can influence glucose levels. Endogenous factors consist of glucagon, the hormone insulin, cortisol, and receptor systems in the muscles and liver. Exogenous factors on the other hand, consist of physical activity and food, namely the type and amount consumed (Firgiansyah, 2016).

The average blood collection time in the morning, namely 80.25 mg /dl, has a more stable glucose level and has the lowest average level compared to the afternoon and evening collection times. This is because the morning is a stable basal state of the body where generally there is not much activity. Choosing the right sampling time can reduce the impact of Circadian rhythm. According to Laila, in 2018 sampling should be done in the morning when preparing the glucose test sample. Ideally, sampling is done at the same time every day, for example, in the morning after fasting overnight. In the morning the levels are lowest and more stable because during the night when the body was asleep, no food was consumed. The results will be more consistent (Gu et al., 2020; Widiantini et al., 2019).

The main benefit of Circadian rhythm is the ability of organisms to adapt to changes in environmental conditions, especially changes in light and darkness due to exposure to sunlight (Prianthara et al., 2021; Segers & Depoortere, 2021). Melatonin is a hormone that signals the body to sleep. Increasing melatonin levels can cause feelings of relaxation, reduce body temperature during sleep, and improve the immune system (Widiantini et al., 2019). The average time for blood sampling during the day, namely 96.5, has a higher average glucose level compared to morning and evening. Every individual has a biological clock pattern, normal people will do many activities during the day and rest at night (Regmi, 2020). Therefore, as a diurnal organism, a person does a lot of activities during the day, such as work, which can make it difficult for the mind and body to relax, which can be a factor in someone experiencing stress. Circadian rhythm through the SCN can regulate the secretion of the hormone cortisol. Excess production of the hormone cortisol can increase during stressful situations, which can counteract the effects of insulin and cause levels high blood sugar. Stress can increase blood sugar levels, so the more stressed a diabetes sufferer is, the more severe their diabetes will be (Ulya et al., 2023).

The increase in average blood glucose levels during the day can be caused by stress factors, which when a person is stressed can increase the hormone cortisol. The

cortisol hormone or stress hormone is a corticosteroid hormone excreted by the adrenal cortex which has the function of increasing gluconeogenesis and reducing glucose use by cells and causing an increase in glucose levels. (Handayani et al., 2023) One of the main factors that increases cortisol secretion in the body is stress. Stress can stimulate the hypothalamus to activate the sympathetic nervous system, releasing Corticotropin Releasing Hormone to stimulate the release of adrenocorticotropic substances and cortisol to trigger the release of vasopressin. Apart from that, the diurnal system also influences cortisol secretion, namely the highest levels when starting activities and the lowest when resting or at night (Sri et al., 2021).

In line with Nugroho's research, it shows that in diabetes sufferers there is a correlation between stress levels and glucose levels in Sukoharjo. When stressed, hormones that increase blood sugar levels are epinephrine, cortisol, glucagon, ACTH (adenocorticotropin), corticosteroids, and thyroid. The hypothalamic-pituitary-adrenal system activates the neuroendocrine system and the sympathetic nervous system due to physical and mental stress (Berkat Br Nababan et al., 2018). According to Ahmed, 2012 although physical activity before meals can influence postprandial insulin action and glucose tolerance, research that has been conducted shows that postprandial glucose spikes are significantly lower at breakfast than at lunch or dinner supporting our case of decreased diurnal glucose tolerance (Saad et al., 2012).

It can be concluded that the average blood glucose level during the day is higher than in the morning. However, respondents number 7 and 9 had lower daytime glucose levels compared to the morning. This is because before the blood collection was carried out, the respondent did sports activities, namely cycling. A study conducted by Shenoy, 2010 showed that aerobic exercise can reduce blood sugar by 37%. It takes more fuel for the muscles to keep working during exercise. In addition, various body reactions will occur, including circulation, metabolism, hormonal regulation, and autonomic nervous structure. Glucose and free fatty acids are energy sources for exercise. Muscle glucose is first used from muscle glycogen reserves, then from blood glucose (Fanana Mahdia et al., 2018).

The average time for blood sampling carried out in the evening was 85.91 mg /dl and after statistical testing there was no significant difference in the average compared to the time for sampling in the morning but there was a slight increase. This can be caused at night when someone begins to enter the rest phase and someone reduces their activity more at night. Glycemic homeostasis also fluctuates throughout the day, and appears to differ in the morning and evening. The decrease in insulin sensitivity and glucose oxidation at night is caused by higher levels of postprandial free fatty acids at night compared to the morning (Nakamura et al., 2021). However, respondents number 3, 4, and 8 had lower glucose levels during the morning compared to the morning. This was influenced by the researchers taking a long time to separate the samples which could influence the results of the examination. Research conducted by Novie, 2020 showed that samples with long delay variations decreased significantly due to glycolysis of blood tube cells (Trisyani et al., 2020)

Conclusion

Based on research result, it can be concluded that the average blood glucose level in the morning in normal people is 80.25 mg /dl, during the day it is 96.5 mg/dl, and at night it is 85.91 mg/dl. Based on the *paired t-test* statistical test, the value *Pair 1* is obtained *Sig. (2-tailed)* 0.010 < 0.05, there is a difference in the average glucose results at the time of sampling in the morning and afternoon and *Pair 2 Sig. (2-tailed)* 0.115 > 0.05, there is no difference in the average glucose test results when sampling in the morning and evening.

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