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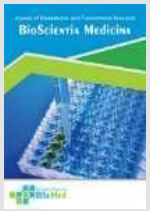

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Differences in Cerebrospinal Fluid Glucose Levels in Meningitis Patients Based on Examination Time: A Single Center Observational Study at Dr. M. Djamil General Hospital, Padang, Indonesia

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A B S T R A C T

Background: Cerebrospinal fluid (CSF) is a vital fluid in the central nervous system that can provide important diagnostic information, especially in cases of meningitis. CSF glucose is one of the key parameters that can help in the diagnosis and monitoring of the patient's condition. However, the stability of glucose in the CSF after sampling is of concern due to the potential for levels to decrease over time. **Methods:** This cross-sectional analytical observational study was conducted at Dr. M. Djamil General Hospital Padang between May and August 2023. CSF samples from patients with suspected meningitis were checked for glucose levels at three time points: the first 1 hour, 2 hours, and 4 hours after sample collection. Patients with puncture trauma and late delivery of samples (more than 30 minutes) were excluded. **Results:** This study involved [number of patients] CSF samples that met the inclusion criteria. The mean decrease in CSF glucose levels between the 1-hour and 2-hour examination was 5.667 mg/dL (±0.75 mg/dL), while between 1 hour and 4 hours, it was 12.183 mg/dL (±1.549 mg/dL). Statistical analysis showed significant differences ($p < 0.001$) between the three examination time points. **Conclusion:** Glucose levels in the CSF of meningitis patients decreased significantly over time after sampling. This emphasizes the importance of checking CSF glucose as soon as possible to obtain accurate results and avoid misinterpretation in the diagnosis and treatment of meningitis.

1. Introduction

Meningitis, inflammation of the meninges or protective lining of the central nervous system (CNS), is a serious medical condition that can be fatal if not treated quickly and appropriately. The meninges consist of three layers of protective membrane that surround the brain and spinal cord, namely the dura mater (outermost layer), arachnoid mater (middle layer), and pia mater (innermost layer). Inflammation of the meninges can be caused by various infectious agents, such as bacteria, viruses, fungi, or parasites, as well as non-infectious factors such as autoimmune reactions, malignancy, or trauma. Meningitis is a significant global public health threat. The World Health Organization (WHO) estimates that meningitis causes around 250,000 deaths each year worldwide. The highest death rate occurs in children under five years of age, especially in developing countries with limited access to health services and vaccinations. Acute bacterial meningitis, the most severe form of

meningitis, has a high mortality rate of 10-15% even with appropriate antibiotic therapy. Apart from that, meningitis can also cause long-term complications such as neurological disorders, deafness, seizures, and developmental disorders in children. Rapid and accurate diagnosis of meningitis is essential to initiate appropriate therapy and improve the patient's prognosis. One of the most important diagnostic procedures in evaluating patients with suspected meningitis is cerebrospinal fluid (CSF) analysis. CSF is a clear fluid that fills the subarachnoid space around the brain and spinal cord. The CSF functions as a protective cushion, provides nutrients, and removes waste products from the CNS. CSF analysis involves taking a fluid sample through a procedure called a lumbar puncture or spinal tap. The CSF sample is then examined in the laboratory to analyze various parameters, including the number and type of white blood cells, protein levels, glucose, and the presence of infection-causing microorganisms. CSF analysis can provide valuable information about the etiology of meningitis, differentiating between bacterial, viral, or fungal meningitis, and monitoring response to therapy.[1-3]

One of the key parameters in CSF analysis is glucose levels. Glucose is the main energy source for brain cells and the nervous system. In healthy individuals, glucose levels in the CSF usually range between 50-80 mg/dL, or around 60-70% of blood glucose levels. However, in meningitis, especially those caused by bacterial infections, CSF glucose levels can decrease significantly. This happens because the bacteria that cause meningitis consume glucose as an energy source for their growth and reproduction. In addition, inflammation of the meninges can also interfere with glucose transport from the blood to the CSF. Low CSF glucose levels (<40 mg/dL) are a strong indicator of bacterial meningitis. However, normal or high CSF glucose levels do not always exclude the possibility of meningitis. In viral meningitis, CSF glucose levels are usually normal or slightly decreased. Non-infectious conditions such as tuberculous meningitis, fungal meningitis, or malignant meningitis can also cause a decrease in CSF glucose levels, although not as severe as bacterial meningitis. Although CSF glucose is an important biomarker in the diagnosis of meningitis, the stability of glucose in CSF samples after collection is a major concern. Some studies have shown that glucose levels in CSF can decrease over time after sampling, even if the sample is stored at the appropriate temperature. This decline can be caused by various factors. The process of glucose metabolism by white blood cells and other cells in the CSF. The bacteria or fungus that causes meningitis may continue to consume the glucose in the CSF sample after collection. Glucose can degrade spontaneously to lactic acid and other products over time. A decrease in CSF glucose levels after sampling can lead to misinterpretation of results and potentially delay appropriate diagnosis and therapy. Therefore, it is important to understand the dynamics of CSF glucose changes over time and determine the optimal time for CSF glucose examination after sampling.[4-7] This study aims to evaluate changes in glucose levels in the CSF of meningitis patients over time after sampling.

2. Methods

This study used an analytical observational design with a cross-sectional approach. This design was chosen because it allows observation and measurement of related variables (CSF glucose levels) at a certain point in time, namely when the patient is suspected of meningitis and the CSF sample is sent to the laboratory. A cross-sectional approach is suitable for the purpose of this study, namely to identify differences in CSF glucose levels at various time intervals after sampling. This research was conducted in the central laboratory of Dr. M. Djamil General Hospital Padang, a tertiary referral hospital in the West Sumatra region, Indonesia. This laboratory is a diagnostic service center that provides various types of laboratory examinations, including cerebrospinal fluid (CSF) analysis. Selection of Dr. M. Djamil General Hospital Padang as a research location was based on several considerations. Dr. M. Djamil General Hospital

Padang is a hospital with a high volume of patients, including cases of suspected meningitis. This allows the collection of a sizable CSF sample in a relatively short period of time. Central laboratory of Dr. M. Djamil General Hospital Padang is equipped with adequate equipment and supplies to carry out CSF analysis, including checking glucose levels. This laboratory is supported by competent experts in the field of laboratory analysis, including laboratory analysts who are trained and experienced in carrying out CSF examinations.

The target population in this study were all patients who were suspected of meningitis and underwent CSF examination at Dr. M. Djamil General Hospital Padang. Patients suspected of meningitis are patients who present with clinical symptoms that suggest meningitis, such as fever, severe headache, stiff neck, nausea, vomiting, and decreased consciousness. The accessible population is all patients who are suspected of meningitis and whose CSF samples are sent to the central laboratory of Dr. M. Djamil General Hospital Padang during the research period, namely between May and August 2023. The research samples were all CSF specimens from patients with suspected meningitis who met the inclusion and exclusion criteria. The inclusion criteria are patients of all ages and genders who are suspected of meningitis based on clinical symptoms and/or physical examination as well as CSF samples sent to the central laboratory in less than 30 minutes after sample collection. Meanwhile, the exclusion criteria are patients with a history of lumbar puncture trauma, namely the presence of blood in the CSF sample due to injury to blood vessels during the lumbar puncture procedure. Puncture trauma can interfere with the interpretation of CSF examination results, including glucose levels and CSF samples that are sent to the laboratory too late (more than 30 minutes after sample collection). Delays in delivery may result in changes in CSF composition, including decreased glucose levels.

CSF samples are taken through a lumbar puncture procedure carried out by a doctor or trained medical personnel. Lumbar puncture is performed by inserting a sterile needle into the subarachnoid space (the space between the arachnoid meninges and the pia mater) at the level of the L3-L4 or L4-L5 lumbar vertebrae. The cerebrospinal fluid that comes out through the needle is collected in a sterile tube. After taking the sample, the tube containing CSF was immediately sent to the central laboratory of Dr. M. Djamil General Hospital Padang. The sample is placed in a special container that maintains the temperature and stability of the sample during transportation. Sample delivery times were recorded to ensure that samples arrived at the laboratory in less than 30 minutes after collection. Examination of CSF glucose levels was carried out at three-time points after sample collection. The first examination is carried out as soon as possible after the CSF sample arrives at the laboratory, ideally within 1 hour after sampling. The second examination was carried out 2 hours after sampling. CSF samples were stored at appropriate temperatures (2–8°C) during this time interval to minimize changes in CSF composition. The third examination was carried out 4 hours after sampling. The CSF samples remained stored at the appropriate temperature during this time interval.

CSF glucose levels were measured using an enzymatic method. This method was chosen because it has several advantages. The enzymatic method is very specific for glucose, so it is not affected by the presence of other substances in the CSF that could interfere with the measurement. This method has high sensitivity, so it can detect small changes in CSF glucose levels. The enzymatic method provides accurate and precise measurement results. CSF samples are reacted with the glucose oxidase enzyme. This enzyme catalyzes the oxidation of glucose to gluconic acid and hydrogen peroxide (H_2O_2). The hydrogen peroxide formed is then reacted with the peroxidase enzyme and chromogen (dye). This reaction produces colored compounds whose color intensity is proportional to the glucose content in the sample. The color intensity of the compounds formed is measured using a spectrophotometer at certain wavelengths. The measured absorbance was then converted into glucose levels using a standard curve.

Data obtained from measuring CSF glucose levels at three time points will be analyzed using appropriate statistical methods. Data analysis steps include: Data Cleaning: Data is examined to identify and address outliers, missing values, or other data entry errors; Normality Test: Data distribution will be tested using the Shapiro-Wilk or Kolmogorov-Smirnov test to determine whether the data is normally distributed or not. If the data is normally distributed, the MANOVA (Multiple Analysis of Variance) test is used to compare the average glucose levels at the three examination time points. If the data is not normally distributed, the Kruskal-Wallis test is used as a non-parametric alternative to MANOVA. If MANOVA or Kruskal-Wallis tests indicate significant differences between groups, post-hoc tests (e.g. Tukey or Dunn tests) are performed to determine which groups are significantly different. This research was conducted in accordance with applicable research ethical principles. Informed consent was not required because this study used anonymous data and did not involve patient intervention. Patient identities were kept confidential and data were analyzed anonymously. This study does not pose any risk or danger to patients because it only uses CSF samples that have been taken for diagnostic purposes. Research data is stored in a safe and secure electronic format. Access to the data was restricted to researchers involved in the study. Data is stored for a minimum of 5 years after publication of research results, in accordance with the data storage policy of Dr. M. Djamil General Hospital Padang.

3. Results

Results of research on 60 CSF samples with diagnosis and suspicion of meningitis. The parameters examined were CSF glucose levels at 1 hour, 2 hours, and 4 hours at room temperature.

Table 1. Mean CSF glucose levels.

Inspection time	Average mg/dL	Lower limit mg/dL	Upper limit mg/dL
1 hour	57,750	50,529	64,971
2 hours	52,083	44,920	59,246
4 hours	45,567	38,454	52,680

The average CSF glucose examination within 1 hour was 57,750 mg/dL (±7,221 mg/dL), the average 2-hour CSF glucose examination was 52,083 mg/dL (±7,163 mg/dL) and the average 4-hour CSF glucose examination was 45,567 mg/dL (± 7.113 mg/dL). This within-subject difference was statistically significant ($p<0.001$).

Table 2. Comparison of mean decrease in CSF glucose levels (mg/dL).

Inspection time		Average mg/dL	Lower limit mg/dL	Upper limit mg/dL	p-value
1 hour	2 hours	5,667	4,917	6,416	0,001
1 hour	4 hours	12,183	10,634	13,732	
2 hours	4 hours	6,517	5,622	7,412	

The mean decrease in CSF glucose levels in the 1-hour and 2-hour examination was 5.667 mg/dL (±0.75 mg/dL) and the average decrease in CSF glucose levels in 1-hour and 4 hours was 12.183 mg/dL (±1.549 mg/dL). in this subject was statistically significant ($p<0.001$). MANOVA was carried out to analyze changes in CSF glucose over time and there was a significant decrease in CSF glucose examination ($p<0.001$).

4. Discussion

The results of this study showed a significant decrease in glucose levels in the cerebrospinal fluid (CSF) of meningitis patients over time after sampling. This decrease was clearly visible at 2 hours and 4 hours after sampling, with a statistically significant difference compared to glucose levels in the first 1 hour. This finding is in line with several previous studies which also reported a decrease in glucose levels in the CSF after sampling. One study showed an average reduction of 3.43 mg/dL per hour in bacterial meningitis patients. Another study reported a greater reduction in patients with tuberculous meningitis. One of the main factors contributing to a decrease in glucose levels in the cerebrospinal fluid (CSF) in meningitis patients is the consumption of glucose by the cells and microorganisms within them. In meningitis, both body cells and the pathogens that cause infection actively utilize glucose as an energy source to carry out various physiological and pathological processes. In response to a meningitis infection, the body will send white blood cells, especially neutrophils and lymphocytes, to the subarachnoid space (where the CSF is located) to fight the pathogens that cause the infection. These white blood cells require large amounts of energy to carry out various activities, such as phagocytosis (engulfing and destroying pathogens), production of cytokines (inflammatory signaling molecules), and migration to the site of infection. Glucose is the main energy source for white blood cells. Through the process of glycolysis, glucose is broken down into pyruvate molecules which then enter the citric acid cycle to produce ATP (adenosine triphosphate), which is the main energy carrier molecule in cells. The more active the white blood cells are in fighting infection, the greater their need for glucose.[8-10]

Apart from body cells, pathogenic microorganisms cause meningitis, such as bacteria *Streptococcus pneumoniae*, *Neisseria meningitidis*, or mushrooms *Cryptococcus neoformans*, which also utilize glucose as an energy source for growth and development. These microorganisms have multiple metabolic pathways that allow them to break down glucose and produce the energy necessary for replication, synthesis of cellular components, and production of virulence factors. The rate of glucose consumption by pathogenic microorganisms can vary depending on the type of pathogen, the number of pathogens present, and environmental conditions in the CSF. In bacterial infections, for example, glucose consumption tends to be higher because bacteria generally have a faster metabolic rate compared to fungi. High glucose consumption by white blood cells and pathogenic microorganisms simultaneously can cause a significant decrease in glucose levels in the CSF. This is especially true in cases of acute bacterial meningitis, where a strong inflammatory response and large numbers of bacteria can cause a drastic drop in glucose levels in a short period of time. Glucose is the main energy source for the brain. A significant decrease in glucose levels can disrupt brain function and cause various neurological symptoms, such as decreased consciousness, seizures, and cognitive impairment. Low glucose levels in the CSF are one of the diagnostic criteria for bacterial meningitis. However, if the CSF examination is delayed and the glucose level has decreased significantly, the examination results can provide an inaccurate picture and complicate the diagnosis. A decrease in glucose levels in the CSF can indicate a severe infection and increase the risk of complications, such as permanent brain damage, hydrocephalus (fluid buildup in the brain), and sepsis (systemic infection). Therefore, understanding the mechanisms of glucose consumption by cells and microorganisms in the CSF is very important for an accurate and timely interpretation of CSF glucose examination results, as well as for optimal clinical decision-making in the management of meningitis patients.[11-13]

The blood-brain barrier (BBB) is a complex structure consisting of endothelial cells lining blood vessels in the brain, pericytes, astrocytes, and the basement membrane. The main function of BBB is to protect the brain from harmful substances in the blood and maintain homeostasis of the brain

environment. BBB selectively regulates the movement of molecules between blood and cerebrospinal fluid (CSF), including glucose. Under normal conditions, BBB has low permeability to glucose, so glucose can only enter the brain through a specific active transport mechanism. However, in meningitis, inflammation that occurs in the meninges (the protective membrane of the brain and spinal cord) can disrupt the integrity of the BBB and increase its permeability. Several mechanisms may contribute to increased BBB permeability in meningitis. Pro-inflammatory cytokines released during the inflammatory response in meningitis can activate cerebral vascular endothelial cells. This activation causes changes in the structure and function of endothelial cells, including increased expression of adhesion molecules and widening of intercellular gaps (tight junctions), which facilitates the movement of molecules across the BBB. Pericytes are contractile cells that surround endothelial cells and play an important role in maintaining the integrity of the BBB. In meningitis, pericytes can become dysfunctional due to exposure to pro-inflammatory cytokines or direct damage by pathogens. Pericyte dysfunction can lead to increased BBB permeability. The extracellular matrix (ECM) is a complex network of proteins and carbohydrates that surrounds BBB cells. ECM plays an important role in maintaining the integrity of the BBB. In meningitis, the ECM can be damaged due to the activity of proteolytic enzymes released by inflammatory cells or pathogens. Damage to the ECM can lead to increased BBB permeability.[14-16]

Increased BBB permeability in meningitis allows glucose to escape from the CSF into the surrounding brain tissue via a passive diffusion mechanism. Glucose will move from areas of high concentration (CSF) to areas of low concentration (brain tissue) until it reaches balance. This process can cause a significant decrease in glucose levels in the CSF, especially if inflammation in the meninges is severe and BBB permeability increases drastically. Decreased CSF glucose levels may contribute to impaired brain function, diagnostic difficulties, and increased risk of complications in meningitis patients. Increased BBB permeability is one of the important mechanisms explaining the decrease in CSF glucose levels in meningitis. Understanding these mechanisms may help in the development of new therapeutic strategies aimed at protecting BBB and preventing a decrease in CSF glucose levels in meningitis patients. Some microorganisms that cause meningitis can interfere with the glucose transport mechanism across the blood-brain barrier, thereby reducing the availability of glucose in the CSF. Decreased glucose levels in the CSF have important clinical implications in the diagnosis and treatment of meningitis. Low glucose levels (<40 mg/dL) are one of the diagnostic criteria for bacterial meningitis. However, if CSF examination is delayed, a decrease in glucose levels can cause inaccurate results and potentially lead to misdiagnosis or delayed treatment. Therefore, the findings of this study emphasize the importance of checking CSF glucose as soon as possible after sampling. Ideally, the examination should be carried out within 1 hour after the lumbar puncture to obtain the most accurate results and reflect the patient's true condition. If testing cannot be performed immediately, CSF samples should be stored at an appropriate temperature (2-8°C) to slow the rate of glucose degradation.[17-20]

This study has several limitations that need to be noted. The cross-sectional design only allows observations at a one-time point, so it cannot provide information about long-term changes in CSF glucose levels. Although the number of samples analyzed was quite large, this study was only carried out in one healthcare center. Therefore, generalization of the results of this study to a wider population needs to be done with caution. This study did not control for several factors that could influence CSF glucose levels, such as the use of antibiotics before lumbar puncture, the severity of the disease, and the patient's nutritional status.

5. Conclusion

This study showed that glucose levels in the cerebrospinal fluid (CSF) of meningitis patients decreased significantly over time after sampling. This decrease was clearly visible at intervals 2 hours and 4 hours after sampling, with statistically significant differences compared to glucose levels in the first 1 hour. These findings underscore the importance of checking CSF glucose as soon as possible after lumbar puncture to obtain accurate and representative results of the patient's condition. Delays in testing can lead to misinterpretation of results and potentially lead to misdiagnosis or delays in treatment.

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