ORIGINAL ARTICLE

Phase angle as a marker for Differentiating Escherichia Coli and Candida Albicans Pathogens

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ABSTRACT

Aim: Early diagnosis is important in bacterial and fungal identification in cerebrospinal fluid (CSF), and treatment option varies according to the identified pathogens. Our aim was to separate bacterial and fungal pathogens with minimum colony number in CSF from sterile CSF by 50kHz phase angle (PA) bioimpedance values.

Methods: We evaluated the performance of the 18 Gauge probe for differentiating bacterial and fungal CSF from sterile. An amount of 200µl sterile CSF was used as a standard in each experiment, and it was inoculated with one and two colony of Escherichia coli (E. coli) and Candida albicans (C. albicans) separately.

Results: PA values of CSF samples were compared with each other concerning one and two colony numbers. It was observed that two colony strains of E. coli and C. albicans could be differentiated from sterile CSF using PA values. One colony of the E. coli and C. albicans strains could not be distinguished from each other due to the small diameter of E. coli. Furthermore, since E. coli has a small diameter, it causes a large extracellular pathway in the environment. Thus, it shows less resistance to the current flow like sterile CSF, so one colony of it has not been differentiated from sterile CSF.

Conclusion: Bioimpedance spectroscopy PA values can provide a predictive approach for differentiating bacterial pathogens from fungal with an inexpensive, simple, and time-saving way in CSF samples with minimal colony number.

Keywords: Bioimpedance, Phase angle, E. coli, C. albicans

INTRODUCTION

Cerebrospinal fluid (CSF) culture is a laboratory examination to look for bacteria, fungi, and viruses in the fluid that flows in the region around the spinal cord. CSF shields the spinal cord and brain from injury, and the examination of it is usually performed with a lumbar puncture. The CSF samples are transferred to the laboratory, and the staff observes if bacteria, fungi, or viruses grow in the dish, and growth indicates an infection. Central nervous system fungal infections present many diagnostic and therapeutic challenges and are associated with a high mortality rate. Candida-related central nervous system infections are usually caused by Candida albicans (C. albicans), which derive from the hematogenous spread and present with meningitis. These cases commonly involve chronic meningitis, brain abscesses, and vasculitis with cerebral infarctions, spinal infections, and mycotic aneurysms. The early initiation of antifungal medication is essential. Escherichia coli (E. coli) meningitis is uncommon in adults, developing mainly as a complication variety of accidental and neurosurgical trauma.[1, 2] The choice of treatment in gram-negative bacillary meningitis depends on the common causative bacteria and the reported efficacy of different antimicrobials that should be modified after culture results.

Bioimpedance spectroscopy (BIS) is based on the electrical properties of body structures. An electrical potential with high frequency passes intra and extracellular spaces; if the potential is applied with a low frequency, it prefers the extracellular fluid.[3] (Figure 1). An increase in the quantity of fluid in the measured compartments decreases the impedance. Bioimpedance methods have been used for tissue differentiation, identifying intraneural needle placement, and tumor detection.[4-7] Studies show that bioimpedance has the potential for clinical utilization in the differentiation of tissue. Kari et al.[8] presented a thin bioimpedance probe needle of standard 22 Gauge size to distinguish between tissues commonly encountered in living organisms. In our study, we prac-

ticed the same technology, but the 18Gauge 1.2x89 mm spinal needle, which connected to the bioimpedance analyzer, was used.

Bioimpedance is simultaneously measured with the probe that was tested to differentiate the bacterial (E. coli) and fungal (C. albicans) type pathogens with minimal colony number in CSF ex-vivo by 50kHz PA values. We hypothesized that if the probe could differentiate the bacterial and fungal pathogens in CSF accurately, it could be used as an early predictive diagnostic tool in the laboratory.

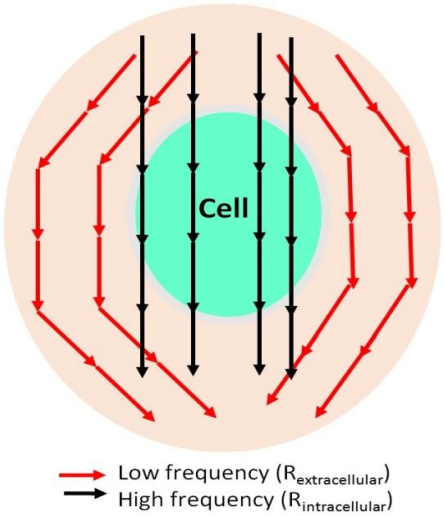

Figure 1: A model of the frequency dependent electrical behavior of the body structure.

MATERIALS AND METHODS

The study was conducted at SANKO University School of Medicine, with the approval of the SANKO University Ethics Committee (2018/10-07). At the Medical Microbiology laboratory, the amount of 200µl sterile CSF was used as standard and was inoculated with one and two colony of E. coli and C. albicans separately. Minimum five 50kHz PA bioimpedance measurements were taken from each specimen within a minute, and their mean values were examined.

Phase angle measurements: Bioimpedance analyzer (Quadscan 4000, Bodystat Inc.) was connected to the 18 Gauge probe. The current was sent to the CSF samples in multiple (5, 50, 100, and 200kHz) frequencies for bioimpedance measurements by using the probe. Many bioimpedance systems utilize 50kHz as a frequency where the capacitor's reactance becomes relatively small so that the current is defined mostly by the resistance. The frequency at 50kHz is one of the most essential and optimal frequency so that we obtained the PA in this frequency. Besides, most published studies have been carried out using devices with a frequency at 50kHz to differentiate structures. Due to the logic of this reasoning, we have chosen to illustrate our PA results only for 50kHz. The PA values were calculated by arctan(reactance/resistance) and were examined by IGOR program (Wavemetrics, Lake Oswego, OR, USA).

Suspension preparation: Bacterial (E. coli) and fungal (C. albicans) strains were used, which were isolated previously from various clinical samples. They were identified by the automated diagnostic system (Vitek2 Compact, Biomérieux, France) and were frozen at -80°C. CSF that was determined sterile after culture evaluation was stored at 2-8°C before using and was used as standard. Microorganisms were cultured onto 5% sheep blood agar (RTA Laboratories, Turkey) to prepare the suspension. The cultures were incubated at 37°C for 24 hours in the CO_2 incubator, and then the microorganisms were transferred from plates to the sterile CSF. In our study, one colony contained 100 microorganisms (100 Colony-Forming Unit (CFU)/ml).

Statistical analysis: IBM SPSS Statistics 23 was utilized for statistical analyses (9). Kruskal-Wallis test was performed for comparison of the CSF samples according to PA values. For pairwise comparisons, the Mann-Whitney U test was used with Bonferroni correction. $P < 0.05$ was considered statistically significant. The p-value was 0.017 for tests with Bonferroni correction.

RESULTS

The PA values of the sterile CSF were found higher than the CSF with bacterial and fungal since the resistance values of sterile CSF were small (Figure 2). Sterile, one, and two colony samples PA values were compared with each other (Table 1). It was observed that two colony strains of E. coli and C. albicans could be differentiated from sterile CSF and each other by using their PA values. However, one colony of the E. coli and C. albicans strains could not be distinguished from each other due to the small diameter of E. coli.

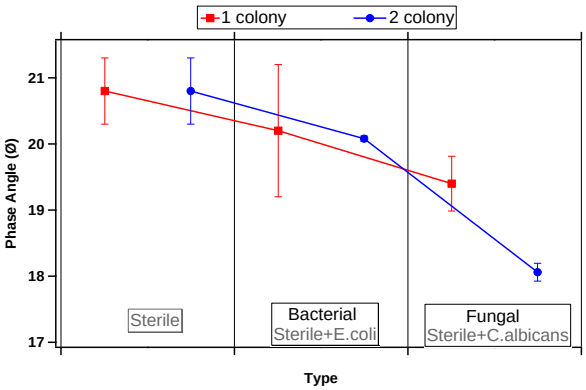

Figure 2: Mean phase angle values in sterile, one and two colony cerebrospinal fluid samples

Table 1: Phase angle median values and their comparison in sterile, E. coli and C. albicans cerebrospinal fluid samples in one and two colony

	Sterile	E. coli	C. albicans	p-value
One colony	20.7 (20.4-21.8)	20.2 (19.7-20.6)	19.2 (19.1-20.2)	<0.005
Two colony	20.7 (20.4-21.8)	20.1 (20-20.1)	18 (17.9-18.3)	<0.001

Phase angle values are expressed as median (minimum-maximum). Analysis of data was performed by Kruskal-Wallis test using SPSS Statistics 23.

Table 2: Pairwise comparison of phase angle values of sterile, E. coli and C. albicans cerebrospinal fluid samples in one and two colony

	Colony	p-value
Sterile-E. coli	1	0.052
	2	0.004
Sterile-C. albicans	1	0.004
	2	0.004
E. coli-C. albicans	1	0.032
	2	0.008

Due to the broad extracellular pathway in the environment of E. coli, it shows less resistance to the current flow like sterile CSF, so one colony of it has not been differentiated from sterile CSF (Table 2).

DISCUSSION

According to the literature, E. coli is a rod-shaped anaerobic bacteria which is gram-negative with 2-3μm long and 0.5μm diameter.[10] Like other biological cells, bacterial cells consist of structures that have various electrical features. The inner composition of a cell is sophisticated and contains vacuoles, mitochondria, nucleus, and many dissolved charged molecules. While bacteria are on average 1-1.5μm in diameter, yeast fungi can be 3-15μm in diameter. Besides candida species are the most common fungal species isolated from nosocomial infections, early diagnosis, and treatment of them are crucial.[11] There are bacterial identification studies that have been performed using BIS techniques.[12, 13] In a study for detecting gram-positive and gram-negative bacteria concentration in drinking water, both the diversity of bacteria and the bacteria concentration had been distinguished by impedance measurements.[14] In other studies, the authors demonstrated that the impedance biosensor was capable of detecting Listeria as low as $1.6x10^2$CFU/mL based on the PA values.[15] In another study, P. aeruginosa was detected by electrical impedance spectroscopy.[16] In studies that used PA to differentiate tissues, it was shown that low PA was associated with the tumor, cell death or decreased cell integrity, but high PA was associated with the healthy cell or cell membrane.[17] Similar to these results, bacterial and fungal CSF PA values were found to be low, and sterile CSF PA values were found high in our study.

We performed a rapid and straightforward bioimpedance method to differentiate sterile CSF from bacterial and fungal with minimum colony numbers. In this research, it was found that sterile CSF, bacterial CSF and fungal CSF with one and two colony numbers resulted in various PA responses. Compared to other methods used in the diagnosis of candida meningitis, the technique used in this study was able to distinguish this pathogen from sterile, even in one colony. However, one colony of the E. coli and C. albicans strains could not be distinguished from each other due to the small diameter of E. coli. Due to the broad extracellular pathway in the environment of E. coli, it shows less resistance to the current flow like sterile CSF, so one colony of it has not been differentiated from sterile CSF. We observed that, when colony number of E. coli and C. albicans were increased to two in CSF, the discrimination of them from sterile became significant since the applied current interacted better with the inner structure of bacteria and fungi. These results indicate that the bioimpedance probe differentiates the sterile CSF from bacterial and fungal CSF samples with two and more colony numbers.

CONCLUSION

The limitation of this study is that the system does not distinguish one colony bacterial concentration in the sterile CSF. The polymerase chain reaction test helps to verify the diagnosis and has become prominent as a rapid diagnostic method in the diagnosis of meningitis. However, these tests are expensive and thus are not being used in routine practice. BIS technique can provide a predictive approach for differentiating bacterial cells from fungal with an inexpensive, simple, and time-saving way in CSF samples with minimal colony numbers. Lumbar punctures procedure should not be performed on anyone with a brain tumor or cyst to prevent brain damage and even death. The probe that we have used in this study has the potential to be used in the rapid discrimination of bacterial and fungal pathogens without collect CSF during the real-time examination.

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