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HASIL PENILAIAN SEJAWAT SEBIDANG ATAU PEER REVIEW
KARYA ILMIAH : JURNAL

Judul Jurnal : Dietary Fat Intake and Changes in Tunica Intima-Media Thickness Internal Carotid Artery in Post Ischemic Stroke Patients

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Kelengkapan Unsur dan Kualitas Penerbit : Dipublikasikan di jurnal ilmiah bereputasi terindex scopus Q4; SJR: 0,114

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Nilai Total = (100%)	30			22.50
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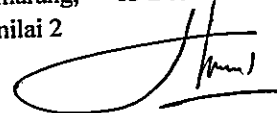
Kelengkapan dan Kesesuaian Unsur : sebagai penulis pendamping (2 orang) pada artikel penelitian dalam jurnal internasional Q4, SJR 0.11, H index 7; abstrak tipe terstruktur, proporsi metode panjang, kesimpulan melebar; unsur abstrak dan badan artikel lengkap,

Ruang Lingkup dan Kedalaman Pembahasan :
 lingkup neurologi; pembahasan cukup dalam, menggunakan 14 pustaka

Kecukupan & Kemutakhiran Data & Metodologi : pendahuluan menggunakan 13 pustaka; metode cukup rinci, menggunakan 4 pustaka; 23 pustaka lebih dari 10 tahun dari 31 pustaka.

Kelengkapan Unsur dan Kualitas Penerbit : tidak mencantumkan riwayat review; penulisan daftar pustaka sesuai ketentuan.

Semarang, 13 Desember 2021
 Penilai 2



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Protein Expression of Human Brain Microvascular Endothelial Cells in Response to Meningitic *C. Sakazakii*: in Vitro and in Silico Analyses

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ABSTRACT

Background: Despite the confirmed association between infant meningitis and necrotizing enterocolitis and *C. sakazakii* little known about the pathogenic interaction of *C. sakazakii* with host cells.

Aim: To characterize functional and regulatory proteins that are differentially expressed in Human brain microvascular endothelial cells (HBMEC) in response to meningitic *C. Sakazakii*

Method: Liquid Chromatography with tandem Mass Spectrometry (LC-MS/MS) analysis was performed to the protein extracted from HBMEC in response to meningitic *C. Sakazakii* in serial time course. Bioinformatics analysis was carried out using SCIEX OneOmics cloud processing software and Advaita's iPathway.

Results: In response to the exposure, the LC-MS/MS analysis indicated different changes in 57 proteins: nucleic acid binding proteins, transporter proteins and structural molecule activity proteins. 1, 9 and 29 pathways were significantly affected after 60, 90 and 120 minutes, respectively. In addition, 179, 242 and 490 gene ontology components were found to be significantly enriched. Interestingly, the tight junction pathway, which is most important component regulating to brain damage, was triggered at 120 minutes post-infection with the *C. sakazakii* meningitic strain.

Conclusion: We conclude that the data presented in this study may facilitate a better understanding of this bacterium, which can cause irreparable damage to a new-born baby's brain. This study open up many new avenues into the research concerning this pathogenicity.

Keywords: Proteomic; HBMEC cells; *C. sakazakii*; Meningitis

INTRODUCTION

Cronobacter is an emerging pathogen of concern that is associated with severe and often fatal cases of infant meningitis and necrotising enterocolitis. Although a great deal of research has been conducted over the past decade to shed light on the virulence of *Cronobacter spp.* and elucidate their mechanisms of pathogenicity, survival and genetics, there are still many hidden traits to be revealed¹. Therefore, *in vitro* studies have continued to investigate these phenomena and can potentially lead to better control of this pathogen and minimise its infections in infants, as well as the elderly and other immunocompromised people¹.

Measurement of protein expression has been detected in *Cronobacter spp.* using proteomic tools to explain the adaptation mechanisms of osmotically stressed cells². Virulence of *C. turicensis* was investigated by focusing on proteins characterisation and leading to conclude the involved molecular mechanisms³.

Likewise, another study by histopathological analysis the potential virulence factors at the proteomic level detected⁴. All of the proteomics studies so far have successfully evaluated *Cronobacter spp.* at the protein level; however, the protein expression of infected host cells is a controversial topic that needs to be studied more⁵. Understanding how cells behave in the presence of a pathogen may provide key answers such as signaling during adhesion or a variety of other stressful responses during conditions⁶. Hence, the identification of pathogenic mechanisms is mainly based on the neat description of molecular process and the detailed protein expression profile. Thus, our study aims to investigate the protein expression modifications by proteomic analysis of HBMEC

cells' response to meningitic *C. sakazakii* strain 767 to generate new insight into its pathogenetic mechanism. We opt to determine whole protein profiles of infected HBMEC cells with *C. sakazakii* strain 767 by comparing them to whole protein profiles of uninfected cells and evaluating whether the important proteins are up or down regulated over the exposure time. Further, differentially expressed proteins were classified based on the protein ontology to help us to analyse and understand functional and biological information about the identified proteins.

MATERIAL AND METHODS

Bacterial strain, cell culture and strain exposure: Convenient laboratory practices were monitored for microbes, chemicals, cell culture and operating laboratory devices. The strain was isolated from an eruption of *Cronobacter* meningitis infection⁷. Clinical isolate was selected based on the phenotype analysis and virulence assays of these strains and whole genome sequence availability⁸.

Cell culture: *sakazakii* strain 767 was added to confluent monolayers of HBMEC cells (1×10^6) and incubated for 60, 90 or 120 minutes. Cell lysis, centrifugation, alkylation and reduction and trypsinisation were performed.

Liquid chromatography with tandem mass spectrometry (LC-MS/MS): Samples were concentrated to dryness in a Speed Vac set for aqueous solution evaporation (approximately 30–40 minutes). The samples were re-suspended and moved into a new LC vial (suspension solution: 20 μ L acetonitrile 5% and formic acid 0.1%). Samples were chromatographically separated based on their affinity to a 20 cm C18 column using an

Cytomegalovirus Infection and Glutamic Acid Decarboxylase Antibodies in Type 2 Diabetic patients

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ABSTRACT

Background: At present, whether human cytomegalovirus (HCMV) infection is associated with type 2 diabetes mellitus (T2DM) is debatable.

Aim: To obtain information about the prevalence of CMV IgG antibodies among type 2 diabetic patients and to identify age, gender, and laboratory differences.

Methods: This cross-sectional study was carried out among DM type2. Seven hundred eighty-three sera type 2 diabetic patients were tested for the presence of HCMV-IgM&IgG antibodies, GAD, and cytomegalovirus DNA by PCR. Chi-square test (χ^2 -test) was applied for testing the significant association between different variables.

Results: The study included 783 diagnosed cases of type 2 diabetic mellitus, 261 of them (33%) were HCMV-IgG positive. Further investigations of HCMV-IgG seropositive samples revealed that 121(46%) tested positive to HCMV-IgM with a significant statistical differences of HCMV IgM within gender ($P=0.02$) and age groups ($P<0.001$). PCR test for diagnosis of HCMV showed that 81(31%) of samples were positive. Searching for presence of GAD antibodies in all HCMV IgG positive samples revealed that 100(38%) of patients were tested positive, however, statistically there was no significant association between positive anti-GAD antibodies with age groups and gender ($P>0.05$).

Conclusion: Our study determined that the higher prevalence of HCMV IgG in DM2 patients, depending on the results of IgM & PCR testes, more than one-third of HCMV IgG seropositive were having recent HCMV infection, about one-third of type two diabetic patient were tested positive to anti-GAD antibodies.

Keywords: HCMV, type 2 DM, anti-GAD antibodies,

INTRODUCTION

The worldwide prevalence of type 2 diabetes mellitus (T2DM) is estimated to have multiplied over the past decades and now includes rapidly increasing numbers of younger age groups. The condition is considerably more complex with much earlier onset, since there is a higher overall risk of life-time complications [1]. T2DM prevalence has risen faster in low and middle-income countries than in high-income countries². Cytomegalovirus (CMV) is a virus found around the world. It is a ubiquitous beta-herpes virus and herpesviridae that infects the majority of humans³. It is a common virus that infects people of all ages. CMV infection is commonly asymptomatic in healthy individual, but can cause severe disease in immunocompromised children or adults and in newborn. A person can also be re-infected with a different strain (variety) of the virus⁴. Active CMV infection or reactivation from a latent state is considered potentially a cofactor for inflammatory disease [5]. CMV may accelerate immune responses by prompting the accumulation of late-differentiated CD8+ and CD4+ T-cells which produce pro-inflammatory cytokines and thereby create a more pro-inflammatory background that might be causing T2DM onset⁶. A Glutamic Acid Decarboxylase (GAD) is an enzyme that present in the pancreas and the nervous system, its aids the body produce a specific neurotransmitter called gamma-aminobutyric acid (GABA), which is an amino acid that decreases the extent of communication to and from the nerves. GAD can also trigger the immune system to produce autoantibodies

against healthy cells⁷. GAD antibodies test is helping to discover whether someone has either Latent Autoimmune Diabetes of Adulthood (LADA) or type 1 diabetes. The test is done to define which type of diabetes someone has. It is particularly useful for adults more than 30 years who get diabetes where diagnosis of T2DM is uncertain⁸. This research aims to obtain information about the prevalence of CMV IgG antibodies among type 2 diabetic patients and to identify age, gender, and laboratory differences.

MATERIALS AND METHOD

This was a cross-sectional study that conducted in diabetes and endocrine gland center in AL-Sader teaching hospital in Al-Najaf Province during the period from January to July 2019. The cases were ascertained as type 2 DM and checked through their hospital records. All type 2 diabetic patients attended the diabetic outpatient clinic during the period of the study were included.

We tested seven hundred eighty-three sera from type 2 diabetic patients aged from 20 to 70 years, two hundred sixty-one of them were found positive for HCMV. IgG sero positive samples were collected to determine glutamic acid decarboxylase (GAD) antibodies and HCMV IgM antibodies. Anti-GAD antibody and specific anti-cytomegalovirus antibody (IgG and IgM) were detected by the ELISA test kit. (BioCheck, Inc.). Wholly blood samples with EDTA were detecting cytomegalovirus DNA. DNA extraction kit and PCR amplification kit are (DNA –Sorb-B) where supplied by Sacaca Biotechnology, Italy. DNA isolation and PCR amplification and thermocycling