

# The Impact of Microbiota and Associated Blood Tryptophan Metabolites on Pain Perception in Patients Undergoing Lumbar Disc Herniation Surgery

## Study Follow-Up Table

Study starting from January 12, 2022, following ethical approval:

1. Ethical approval from the DEU Non-Interventional Ethics Committee is estimated to be obtained in 2022.
2. Commencement of patient data recording following the approval of the Ethics Committee.
3. Regular maintenance of records in the SPSS computer program for the purpose of statistical analysis of the acquired data.
4. Estimated completion of results by November 2022.
5. Conducting statistical analysis after data collection.
6. Evaluation of the correlation between tryptophan metabolites (picolinic acid, 3-Hydroxykynurenine, anthranilic acid, kynurenine, quinolinic acid, kynurenic acid, xanthurenic acid) routinely collected in blood samples after lumbar disc herniation surgeries and Visual Analog Scale (VAS) scores.

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Ethic committee decision dated 31.08.2022, numbered 7131-GO

# Study Protocol

## 1. Introduction

The human body is closely associated with a complex ecosystem known as the microbiota. The microbiota encompasses the total of microorganisms found in various regions of the body, from the digestive system to the skin and respiratory system. These microorganisms can have significant effects on health and disease, and recent research indicates that the gut microbiota, in particular, plays a decisive role in pain perception.[1] [2]

In the past decade, research on the influence of gut microbiota on pain has been increasing.[3] The gut microbiota interacts not only with the digestive system but also with the nervous and immune systems.[4] These interactions may modulate pain mechanisms and contribute to chronic pain. Recent research suggests that gut microorganisms may regulate brain function and behaviour.[5] This has been considered an exciting development in the field of anesthesia, because the effect of intestinal microbiota on pain perception offers a new perspective in the development of anesthesia practices.

Tryptophan can be converted into various metabolites by the gut microbiota, and the effects of these metabolites on pain are being investigated. [6] The effect of the gut microbiota on tryptophan metabolism is an important mechanism in regulating pain perception. [7], [8]

An important feature of the microbiota is that the signaling molecules it produces through Tryptophan metabolism can affect pain mechanisms. Some bacteria, especially members of the microbiota such as *Escherichia Coli*, can produce tryptophan.[9] Furthermore, commensal bacterial species that produce AhR ligands play an important role in regulating AhR activity. [10], [11]. The ability of these bacteria to metabolize Tryptophan can lead to the production of the neurotransmitter tryptamine. [12].

Pain is a subjective experience that cannot be directly observed or measured by biological tests. Therefore, the assessment of pain is largely based on the individual's own subjective interpretation. Although its adequacy in pain assessment is questioned, the method used is generally Visual Analogue Scale (VAS).

In this study, we aimed to examine the effect of microbiota and tryptophan metabolites on pain perception in patients undergoing lumbar disc herniation surgery. We evaluated how the gut microbiota affects pain mechanisms by measuring levels of tryptophan metabolites and assessing pain severity in the pre- and postoperative periods.

## 2. Materials and Methods

In this study, we adhered to the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines to ensure the quality and transparency of the reporting of our observational study.

Our study includes 37 lumbar disc herniation (LDH) surgeries performed in Dokuz Eylül University Faculty of Medicine Central Operating Rooms Brain and Nerve Surgery Hall. For the blood samples in which we evaluated tryptophan metabolites; 2 mL of blood was used from the blood samples taken during routine surgery preparation for the patients and from the routine blood samples taken in the postoperative period, and no additional invasive intervention was performed. We elucidated the relationship between tryptophan metabolites (Picolinic Acid, 3-Hydroxykynurenine, Anthranilic Acid, Kynurenine, Quinolinic Acid, Kynurenic Acid, Xanthurenic Acid) and their variations due to microbiota alterations with VAS in acute pain. Preoperative, postoperative, 8th-hour, and 24th-hour blood samples were routinely collected from patients, with 2 ml of sample material used each time. Concurrently, pain information and vital signs were recorded. After the necessary centrifugation processes, the tryptophan metabolites were analyzed by the relevant laboratory team.

In VAS assessment, patients are typically asked to choose a value between "0, no pain" and "10, the worst imaginable pain." VAS is an 11-point scale (ranging from 0 to 10) that allows patients to numerically express the intensity of their pain.[13]

### 2.1. Data Collection

Blood samples were planned and collected from patients during the preoperative evaluation, at the 8th postoperative hour, and at the 24th postoperative hour, adhering to clinical routines for these procedures. Concurrently with each blood draw, the patient's VAS score, the nature of the pain, and vital signs were recorded. At the end of the surgery, procedures were performed in the recovery unit according to the protocols, and the same data along with vital signs were recorded. Blood samples collected from the patients were centrifuged at 4000 rpm for 10 minutes, after which the serum and plasma portions were transferred to Eppendorf tubes. The blood samples were stored at -80°C for preservation. For the same patients, 24-hour postoperative blood collection was performed, and similar data, including VAS scores, were recorded.

## 2.2. Inclusion and Exclusion Criteria for Case Selection

### Inclusion Criteria:

- Patients undergoing lumbar disc herniation surgery at Dokuz Eylül University Hospital.
- Patients who have read and consented to the informed consent form for the study.
- Age 18 years or older.
- Surgery duration exceeding 2 hours.

### Exclusion Criteria:

- Patients unwilling to participate in the study.
- Surgery duration less than 2 hours.
- Patients under the age of 18.
- Patients with a history of abdominal surgery.
- Patients who received antibiotic therapy for intestinal pathology within the last 3 weeks.
- Patients with known abdominal pathologies that are suspected to alter the microbial flora (e.g., Crohn's disease, ulcerative colitis, celiac disease).

Patient records, clinical histories, examination findings, and relevant laboratory results were electronically recorded. All these data were documented on a prepared data collection form and transferred to an electronic database.

## 2.3. Studying samples collected from patients

Sample analyses were performed using an Ultivo tandem mass spectrometer (6465B, Agilent Technologies, Santa Clara, CA, USA) connected to an Agilent HPLC system. The analyses of kynurenine pathway metabolites were conducted using the CE-IVD certified and validated commercial Jasem Kynurenine Pathway Metabolites LC-MS/MS analysis kit (Sem Laboratory Devices Marketing Co., Istanbul, Turkey). The analytical process was completed using the mobile phases (mobile phase A and B), analytical column, calibrators, stable isotope-labeled internal standard (IS) mixture, and sample preparation reagents provided with the commercial kit.

#### 2.4. Sample Preparation for Kynurenine Pathway Metabolite Analysis

Preparation of the calibrator set; 100 µl calibrator was taken into the HPLC vial, 50 µl internal standard was added and diluted with 250 µl Reagent-1 and injected into the LC-MS/MS system. Sample Preparation; 100 µl serum/plasma/control sample was taken into Eppendorf, and 50 µl internal standard was added and vortexed for 5 seconds after protein precipitation with 250 µl reagent-1. It was centrifuged at 4500rpm for 5 minutes. The resulting upper phase was taken into the HPLC vial and transferred to the LC-MS/MS system.

#### 2.5. Ethics

Ethics committee approval for this study was received locally from the University Izmir Dokuz Eylul University Hospital (decision dated 31.08.2022, numbered 7131-GO). The study was conducted following the principles of the Declaration of Helsinki. Since our study was planned retrospectively, approval was obtained from the ethics committee without obtaining informed consent from the patients.

## References

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