Exploring the role of tryptophan metabolites in pediatric migraine

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1. Research objectives, study content and key questions to be addressed

1.1 Research objectivesThis research focuses on the key scientific issues of "tryptophan metabolites andchildhood migraine", and explores the pathogenesis, diagnosis, treatment, andprevention of childhood migraine by combining clinical characteristics and medicalhistory and other multi-level data, with the aim of establishing an assessment systemfor early identification and intervention of childhood migraine, systematicallydeveloping an individualized treatment and assessment system for childhood migraine,and conducting clinical translational research in the field of childhood migraine. Weaim to establish an assessment system for early identification and intervention ofchildhood migraine, systematically carry out individualized treatment and assessmentsystem for childhood migraine, and conduct clinical translational research in the fieldof childhood migraine. The following are the details:The aim of the study is to establish a system for the early identification andintervention of migraine in children, and to systematically develop an individualizedtreatment and evaluation system for migraine in children.

1.2 Research content

1.2.1 Exploration of the pathogenesis of migraine in childrenFor the subjects included in the study, blood was collected at any time during the first 2-4 hours of the headache attack in migraine patients; blood was collected at least 24 hours before and after the inter-episode period without headache attack in migrainepatients. In healthy controls, blood was collected in a quiet state. Tryptophanmetabolite levels were measured by ELISA. The plasma levels of tryptophanmetabolites were analyzed in different groups according to the collected medicalhistories, and the plasma levels of migraine patients were compared with those ofhealthy controls by t-test: one-way ANOVA was performed between different types ofmigraine: correlation analysis was performed between the frequency, duration,duration, severity and two types of migraine attacks; logistic regression wasperformed to screen for possible factors. The results of the above analyses werecombined to explore the pathogenesis of migraine in children and to lay thefoundation for the improvement of the diagnosis of migraine in children and thedevelopment ofnew drugs.

1.2.2 Exploration of specific diagnostic indexes for migraine in childrenFor the subjects included in the study, blood was collected at any time during the first 2-4 hours of the headache attack in migraine patients, and blood was collected at least 24 hours before and after the headache attack in the inter-episode period in migrainepatients. Tryptophan mctabolite levels were measured by ELISA. The ROC curve wasused to analyze the diagnostic value of the combined diagnosis of tryptophanmetabolites, and tryptophan metabolites, and the area under the curve (AUC) oftryptophan metabolites and the combined diagnostic indexes of both were subjected to independent sample t-test to analyze whether there was a statistical differencebetween the three, and the diagnostic test evaluation indexes were used to evaluate thecombined diagnosis of tryptophan metabolites, and tryptophan metabolites.respectively, and to evaluate them as The possibility and accuracy of the

specificdiagnostic index ofmigraine in children were evaluated.

2. The proposed research method, technical route, experimental protocol andfeasibility analysis.

2.1 Experimental protocol

2.1.1 Inclusion and xclusion criteria of study subjects

Case group inclusion criteria:(1) Age 4-18 years old, male and female are not limited. (2) Meet the diagnosticcriteria of ICHD-3 with aura, without aura, and chronic migraine. (3) Migraine wasdiagnosed by two or more specialized neurologists. (4) PedMIDAS score > 11 (5)Migraine had never been treated (6) Patients and families gave informed consent tothe study purpose,significance, risks, benefits, and informed consent. Source: Department of Pediatrics Outpatient and Inpatient Unit, Oilu Hospital.Shandong University

Inclusion criteria for the control group:(1) Age 6-18 years, male and female. (2) Did not meet the diagnostic criteria of ICHD-3 migraine and other headaches. (3) Physical health allowed their participationin the study. (4) Patients and their families gave informed consent to the studypurpose, significance, risks, benefits, and information of the study. Source: Department of Pediatrics Outpatient and Inpatient Unit, Oilu Hospital.Shandong University.

Exclusion criteria:

(1) presence of drug overdose (2) autoimmune diseases (3) neurological diseasesother than migraine, intracranial masses (4) congenital or hereditary diseases (5)diabetes. bronchial asthma, cardiovascular diseases, pulmonary diseases (6) othertypes of primary and secondary headache.

2.1.2 Data collection

(1) Data sources:questionnaires, outpatient medical records andauxiliaryexamination results.

(2) Collection methods: questionnaire survey, medical records review, bloodcollection.

(3) Data composition:Basic personal information, migraine triggering factors, clinical features of headache,medication, past medical history, ancillary examinations, and blood samples werecollected from all enrolled headache patients.

①Basic personal information included namc, gender, agc, height, weight (calculatedBMI) cultural level, residence address, and contact information.

②Migraine triggering factors: genetic factors, endocrine (menstruation), diet (cheese.chocolate, puffed food), psychological factors (sleep, anxiety, depression, emotional stress, etc.

③Clinical characteristics of headache included: whether the patient was in the attackphase or inter-attack phase, the location of the patient's headache, the nature of theheadache, the degree of pain (assessed using the visual analog scale VAS Appendix, the duration of the disease (years), the frequency of the headache, the duration of eachheadache, the time of the last pain, the presence of aura, aura symptoms, concomitantsymptoms (photophobia, phonophobia, nausea, vomiting, gastrointestinal

symptoms),exacerbation and remission The information of each headache, duration of last pain,presence of aura, aura symptoms, concomitant symptoms (photophobia, photophobia.nausca, vomiting, gastrointestinal symptoms), aggravation and relicf factors, familyhistory, etc.
④Also record medication use, past medical history, ancillary test results such asroutine blood, liver function, kidney function, lipids, blood glucose, ECG, TCD, headCT.head MRIhead MRA examination,etc.PedMIDAS scale score, migraine treatment efficacy assessment scale, headachediary
⑤5ml of venous blood

2.1.3 Study method
(1) The trial was reviewed by the Research Ethics Committee of Qilu Hospital of Shandong University. Patients were obtained from the pediatricoutpatient clinic of Qilu Hospital of Shandong University, with written permissionfrom parents or guardians and, where appropriate, consent from the children. Theinvestigators were responsible for design, data collection, analysis, and interpretation. Data were collected by field researchers. and all data were confidential for theduration of the trial; trialists and data analysts could not communicate data with eachother. Eligible healthy children and children with migraine were recruited in the first step and basic information about the children and headache characteristics were obtainedby the site staff, and blood was retained from the study subjects for Elisa assay, respectively.
(2) Method for determination of tryptophan metabolite content:
①Timing of blood collection
In the case group, blood was collected at any time during the first 2-4 hours of themigraine attack; in the inter-migraine attack group, blood was collected at least 24hours before and after the headache-free period. In the healthy control group, bloodwas collected in a quiet state.
②Storage and testing methods
5 ml of elbow venous blood was collected from patients and healthy children inpurple tubes, and the samples were immediately transferred to ice-cold glass tubescontaining a mixture of anticoagulant and protease inhibitor, and centrifuged at 3000rpm for 5 minutes at 4°C. The supernatant was stored at -80°C, and tryptophanmetabolite levels were measured by ELISA, and the case and control groups were compared and analyzed.

2.1.4 Data analysis
Statistical analysis was performed using SPSS26.0 software analysis, and statisticaldata were tested for normality, and the mean standard deviation (x + S) was used formeasurement data that conformed to the normal distribution, and one-way ANOVAwas applied for comparison between the three groups, and t-test for two independentsamples was used for comparison between two groups. The correlation between theplasma levels between migraine attacks and the duration of the disease was analyzedby SPSS26.0 correlation analysis.
(1) After completing the questionnaire survey, the researchers collected data

andanalyzed the risk of migraine in children due to the interaction of multiple factors, andused logistic regression to screen the indicators related to the risk of migraine inchildren due to the interaction of multiple factors, and established an early assessmentmodel of migraine in children to achieve early detection, early diagnosis and earlyprevention of migraine in children.

(2) Based on the collected medical history, different group analyses were performed.and the plasma levels of tryptophan metabolites in migraine patients were comparedwith those in healthy controls by t-test; one-way ANOVA was performed on theplasma levels of tryptophan metabolites between different types of migraine:correlation analyses were performed between the frequency, duration, duration.severity and two types of migraine attacks; and logistic regression was performed toscreen for possible influences on the risk of migraine in children. Logistic regressionwas performed to screen the factors that may influence the diagnosis of migraine inchildren.

(3) To analyze the diagnostic value of tryptophan metabolite and tryptophanmetabolite combined diagnosis by ROC curve, and to analyze whether there is anystatistical difference between the three diagnostic indexes by independent samplet-test, and to evaluate the diagnostic indexes of tryptophan metabolite and tryptophanmetabolite combined diagnosis by diagnostic test, and to evaluate the diagnosticindexes of tryptophan metabolite and tryptophan metabolite combined diagnosis asThe possibility and accuracy of specific diagnostic indexes for migraine in childrenwere evaluated.

2.1.5 Feasibility analysis

(1) The Elisa kit technology and medical statistics technology used in this projecthave been widely and maturely applied in other biomedical research fields, and thisproject has all the core technology reserves that can guarantee the successfulcompletion of the subject.

(2) The PICU of Qilu Hospital of Shandong University, which has rich experience insciсntific rescarch and clinical medicinc, as well as rich clinical cases, is sufficicnt tomeet the needs of scientific research; we have sufficient strength in terms of scientificresearch strength, talent demand and economic support to ensure the successfulcompletion of our experiment.

(3) The pediatric outpatient clinic of Qilu Hospital of Shandong University receives alarge number of children with migraine every day, and the severity of the disease iscomplex and diverse, so the number and diversity of children with migraine aresufficient to meet the needs of our experiment.