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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

THE SIGNIFICANCE OF ABDOMINAL OXIMETRY IN THE DIAGNOSIS AND PROGNOSIS OF NECROTIZING ENTEROCOLITIS IN PREMATURE INFANTS

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GENERAL DESCRİPTİON OF THE WORK

Relevance of the research. The rapid development and modernization of medical technology, along with the organization and improvement of perinatal care based on a three-tiered system, have significantly increased the survival of premature infants in recent years. According to the World Health Organization (WHO), 15 million babies are born prematurely every year in the world, and this figure continues to grow[1]. Perinatal pathologies, mainly resulting from hypoxia, have a high incidence in premature infants[2]. Necrotizing enterocolitis (NEC) is more common in premature infants exposed to perinatal hypoxia[2], its incidence is 1% in premature infants with low birth weight, 11% in those with very low birth weight (<1500 g), and 22% in those with extremely low birth weight (<1000 g) premature infants[3].

Necrotizing enterocolitis in newborns is a life-threatening disease accompanied by inflammation and necrosis of the intestinal wall, mainly found in premature infants. The clinical picture is nonspecific and diverse, its pathophysiology has not been fully studied, but three main risk factors are noted for its development: immaturity, bacterial colonization of the intestines, and formula feeding[4,5].

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Disruption of intestinal wall integrity, including breakdown of tight junctions, represents an early stage of intestinal injury[6].

Alterations in tight junction proteins can lead to the disruption of the mucosal barrier,facilitating the translocation of pathogens and foreign antibodies across the epithelial barrier, which is considered an important step in the pathogenesis of necrotizing enterocolitis[7]. Claudin-3 is a crucial tight junction claudin, and its over-expression in the intestine, small size, and paracellular localization are suitable for early, non-invasive detection of tight junction disruption in urine[8].

Oximetry is widely used as a promising method for assessing hypoxic conditions and evaluating oxygenation in the brain and abdominal cavity[9]. Oximetry is performed by near infrared spectroscopy (NIRS). NIRS has many advantages, including non-invasiveness, ease of use, and real-time analysis of indicators[10]. The diagnostic and prognostic value of abdominal oximetry in necrotizing enterocolitis in premature infants born with hypoxia remains unstudied. All of the above confirms the relevance of the current research work and creates the basis for conducting research in this aspect.

Object and subject of the research. The object of observation of the study was premature infants admitted to the hospital with suspected necrotizing enterocolitis, and the subject of the study was the examination of hypoxic-ischemic lesions of the intestine by abdominal oximetry and intestinal barrier disruption through the detection of claudin-3 for the purpose of early diagnosis and prognosis of necrotizing enterocolitis in these infants.

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The purpose of the study. To determine the informativeness of abdominal oximetry in early diagnosis and prognosis of necrotizing enterocolitis in premature infants.

Research Objectives.

1. To study the risk factors of necrotizing enterocolitis in premature infants;
2. To determine the characteristics of the course and stages of necrotizing enterocolitis in premature infants;
3. To conduct a comparative analysis of abdominal and cerebral oxygenation levels in premature infants;
4. To examine the role of tight junction protein - claudin-3 in diagnosis of NEC among premature infants exhibiting clinical suspicion of necrotizing enterocolitis;
5. To explore the correlation between cerebral and abdominal oximetry parameters and tight junction protein - claudin-3 in premature infants;
6. To develop a diagnostic and prognostic algorithm of necrotizing enterocolitis in premature infants.

Research methods

- Anamnestic;
- Clinical;
- Laboratory;
- Instrumental examination methods (utilizing techniques such as radiological imaging, ultrasound and oximetry to evaluate the gastrointestinal tract);
- Mathemathical-Statistical Calculation methods.

Research Venue: The scientific research work was carried out in the departments of neonatal intensive care and premature infants of the Scientific Research Institute of Pediatrics named after K.Y. Farajova, Baku Medical Plaza, the Republican Perinatal Center, Maternity Hospital No. 5 named after Sh. Alasgarova, laboratory studies were carried out in the clinical and biochemical laboratories of TET LAB during 2020-2023.

Key propositions of the Dissertation for Defense:

1. The development of inflammatory-necrotic changes in the gastrointestinal tract is characterized by abdominal oximetry parameters among premature infants.

2. The abdominal oximetry examination method is of particular importance in the early diagnosis and prognosis of necrotizing enterocolitis in premature infants .

3. The state of mesenteric blood flow and the level of intestinal defense mechanisms determine the development and outcome of necrotizing enterocolitis in premature infants.

4. The diagnosis and prediction of necrotizing enterocolitis is possible through an algorithm created based on the determination of oximetric parameters and tight junction proteins.

Scientific novelty of the study.

- The comparative role of anamnestic, clinical, laboratory and instrumental examinations was studied in the early diagnosis of necrotizing enterocolitis in premature infants, and the characteristics of the association of risk factors with the development of NEC was analyzed.
- The relationship between the state of mesenteric blood flow and intestinal defense, also, the relationship of oximetric indicators and tight junction proteins which reflect the intestinal defense with hematological indicators was determined; oximetric criteria were determined to assess the relationship between cerebral and intestinal ischemia.
- The significance of near-infrared spectroscopy and claudin-3 indicators in the early diagnosis of necrotizing enterocolitis in premature infants, the prognostic role of oximetric parameters were determined; the prognostic significance of lactate levels in predicting fatal outcomes was identified.
- An algorithm has been created for diagnosis and prognosis of necrotizing enterocolitis based on near-infrared spectroscopy parameters, tight junction protein, and lactate levels.

Practical significance of the study:

- determination of claudin-3 levels in urine in combination with a non-invasive, easy-to-perform near-infrared spectroscopy method defines the early diagnosis and prognosis of the development of necrotizing enterocolitis in premature infants, providing timely examination and treatment tactics in practical experience;
- A diagnostic and prognostic algorithm of practical significance which has been prepared for the improvement of early diagnosis and more accurate prognosis of necrotizing enterocolitis in

premature infants will help to reduce NEC-related mortality and complications that may arise during the course of the disease.

Application of the research results in practice. In the course of the work, the results of the research work were applied in the educational process of the Children's Disease I of the Azerbaijan Medical University and in the departments of the Scientific Research Institute of Pediatrics named after K.Y. Farajova.

Approbation of the study. The study results were reported:

International Scientific and Practical Congress "Actual Problems of Pediatrics" (Azerbaijan, Baku, October 11-13, 2022); EIP – Excellence In Pediatrics (Amsterdam, 2022, December 1-3); International Scientific and Practical Congress dedicated to the 100th anniversary of the birth of the National Leader H. Aliyev (Azerbaijan, Baku, May 3-6, 2023); LXXVIII International Scientific and Practical Conference "Modern Medicine. New Approaches and Current Research", Moscow, 2023.

Published works. The main results of the work have been published in 11 journal articles,including 3 articles abroad and 8 articles in Azerbaijan. 5 theses were published on the subject of the thesis,1 of them is abroad.

The dissertation is presented in Azerbaijani on 169 pages (217 950 characters), and consists of an introduction (9800 characters), a literature review (45000 characters), research materials and methods (17500 characters), Chapter III (42000 characters), Chapter IV (26500 characters), Chapter V (25600 characters), Conclusion (49000 characters), Results (1800 characters), Practical recommendations (750 characters), a list of used literature and abbreviations.

211 literary sources were used in the writing of the dissertation, of which 9 were cited from the works of Azerbaijani, 30 from Russian and 172 from foreign scientists.

The dissertation work is illustrated with 24 tables, 18 graphs and 22 pictures.

MATERIALS AND METHODS OF THE RESEARCH

The research work was carried out in 2020-2023 at the Research Institute of Pediatrics named after K.Y. Farajova, the clinical base of

the Children's Disease I of the Azerbaijan Medical University, the Republican Perinatal Center, the Maternity Hospital No. 5 named after Sh. Alasgarova, and the Baku Medical Plaza private medical institution. A medical card was drawn up for each child. The research work was carried out in accordance to protocol No. 18 with the requirements of the Ethics Committee of the Azerbaijan Medical University. 118 premature infants were involved in the study. 30 were conditionally healthy premature infants, and 88 were children with suspicion of necrotizing enterocolitis of these children.

Dynamic observation of 88 premature children with initial suspicion of necrotizing enterocolitis was carried out and, depending on the signs of hypoxic damage to the central nervous system, they were divided into the main (32 children with signs of hypoxic damage to the central nervous system) and comparison (56 children without signs of hypoxic damage to the central nervous system) groups; at the same time, depending on the confirmation of the diagnosis of necrotizing enterocolitis, they were divided into groups A (with a confirmed diagnosis of NEC) and B (with an unconfirmed diagnosis of NEC).

Based on clinical, laboratory and instrumental examinations, the diagnosis of necrotizing enterocolitis was confirmed in 29 of 88 children (group A), and the diagnosis of necrotizing enterocolitis was not confirmed in 59 children (group B). Also, children included in the main and comparison groups were compared in 2 subgroups according to the confirmation of the diagnosis of necrotizing enterocolitis: A – subgroups with a confirmed diagnosis of necrotizing enterocolitis and B – subgroups with an unconfirmed diagnosis of necrotizing enterocolitis.

Thus, in the main group: subgroup IA – 9 children, subgroup IB – 23 children; in the comparison group: subgroup IIA – 20 children, subgroup IIB – 36 children. 60 boys and 58 girls were among of the 118 examined children. 15 children died. 2 of them died in the late and 13 children in the early neonatal period. Of the dead children, 5 (17.2%) had a confirmed diagnosis of NEC, and the other 10 (16.9%) died from a diagnosis of sepsis.

The average gestational age of all children (Mean±SD) – 31.9±2.90 weeks; min 26 weeks; max 36 weeks (Median 32 weeks);

body weight (Mean±SD) - 1621.8±502.6g, min 800 g, max 2500g; height (Mean±SD) – 40.9±4.2cm, min 30 cm, max 48cm.

Conditionally healthy premature infants with Apgar score of more than 5 points and physiological course of the adaptation period were included in control group, who were discharged home with their mothers on the 2nd-3rd day of life.

Inclusion criteria for the study:

- obtaining written consent from parents;
- premature infants with suspicion of necrotizing enterocolitis;
- premature infants (gestational age <37 weeks);
- newborns corresponding to gestational age.

Exclusion criteria for the study:

- term infants (gestational age ≥37 weeks);
- small for gestational age;
- congenital and chromosomal anomalies;
- infants with no suspicion of necrotizing enterocolitis during the first 3 weeks.

Examination methods

The results of anamnestic data, laboratory and instrumental examinations were analyzed according to the complex examination program. The diagnosis of NEC was confirmed based on clinical, laboratory and instrumental examinations.

Laboratory examinations.

Complete blood count and biochemical blood tests, determination of blood gases and electrolytes, and urine and feces laboratory tests were analyzed. Claudin-3 levels were studied in 69 children in urine related to the objectives of the study in order to determine intestinal barrier damage. The research work was carried out in the TET-LAB medical research laboratory, using the ELISA (enzyme linked immunosorbent assay) method and using Human claudin-3 reagent kits from BT LAB.

Instrumental examinations. Ultrasound examination, radiological examination, neurosonography, echocardiography and near infrared spectroscopy (NIRS) examinations were performed in infants suspected of NEC. Tissue oxygenation was measured using near-infrared spectroscopy for diagnostic and prognostic purposes when

initial signs of suspicion of necrotizing enterocolitis appeared in premature infants. Measurements were made by using pediatric somasensors with an oximeter for 2 hours in both- the abdominal (infraumbilical) and frontal (frontoparietal) regions, and continuous pulse oximetry was applied to the patient.

Cerebral and abdominal tissue oxygen saturation (r_cSO_2 and $rsSO_2$, respectively) were measured, and indicators such as SCOR and FTOE were determined.

$FTOE = \frac{(SaO_2 - rSO_2)}{SaO_2}$ (fractional extraction of oxygen from tissues),

SaO_2 -arterial blood saturation

$SCOR = \frac{r_sSO_2}{r_cSO_2}$x100% (splanchnic-cerebral oxygenation ratio)

Statistical processing of the data

Statistical processing of indicators was carried out in the Windows SPSS20 system. In case of normal distribution of indicators, parametric tests were used, and in case of abnormal distribution, non-parametric tests were used. In addition, in order to study the trend, we sometimes gave the mean indicator along with the median indicator.

After the description of the indicators, a comparison was made between unrelated and related groups. If a normal distribution is shown, then unrelated groups were compared using the T-Student test, and if an abnormal distribution is shown, then the Mann Witney U method was used to accept or reject the

Ho hypothesis based on the P value, and the alternative hypothesis was confirmed. The correlation coefficient was calculated to study the relationship between two variables.

The Pearson or Spearman test was used depending on whether the indicators showed a normal or abnormal distribution. Since there was more abnormal distribution in our study, the Spearman test was used more often. In addition, ROC curves of indicators were studied to determine the development of necrotizing enterocolitis. Chi-square test (x^2) was used to compare categorical variables.

RESULTS OF THE INDIVIDUAL STUDY

The role of clinical, laboratory and instrumental examinations in the diagnosis of necrotizing enterocolitis in premature infants

The somatic, pregnancy, obstetric anamnesis of mothers of children suspected of necrotizing enterocolitis, as well as the physical development indicators of the newborn in the early neonatal period, assessment by Apgar scale, clinical examination, instrumental examination results were found out and comparisons were made across groups and subgroups in this half of chapter. While investigating the frequency of children born from pregnancies occurring with in vitro fertilization (IVF) by groups, these children were found to be more common in the comparison group (n=9,16,1%), while in the main group, only 1 child (3.1%) was born with IVF (χ^2 =8,125; p=0.017). Children born with IVF were mainly among those with suspected of NEC (χ^2=3.725,p=0.054). However, there was no association between IVF and the confirmation the diagnosis of the NEC or the outcome of necrotizing enterocolitis, i.e., surgery or death.

Children born from III pregnancy (12%) and more predominated in mothers with a complicated obstetric history (χ^2=14.5, p=0.024) in the group with the infants suspected of NEC. Children from the third and more pregnancies prevailed among those with a confirmed diagnosis of NEC compared to the other subgroup (χ^2=20.7, p=0.004). A complicated obstetric history (previous pregnancies resulting in miscarriage and abortion) was almost twice more in this group of mothers than in the mothers of other groups of children, as 33.3% of mothers of children with a confirmed diagnosis of NEC had a complicated obstetric history, while 16.4% of mothers of children without a confirmed diagnosis had a complicated obstetric history. The birth of a child from the third and more pregnancies increased the development of NEC by 4.5 times (p=0.001).

However, there was not a relationship with the number of birth in children with a confirmed diagnosis of NEC (χ^2=11.20, p=0.082). Also, no relationship was noted between the number of birth and groups and subgroups, as well as between the outcome of NEC (p>0.05).

There was no difference between the groups in terms of multiple pregnancy (χ^2=2.939, p=0.23).

No association was found between groups and subgroups in terms of preeclampsia (χ^2=1.34, p=0.512) and anemia (χ^2=0.072, p=0.965) during pregnancy.

Coagulation disorders were noted in 1 (3.1%) case, hypothyroidism in 1 (3.1%) case in mothers of the main group's children, while in the comparison group, hypothyroidism was noted in 1 case (1.8%), HELLP syndrome in 1 case (1.8%), and cardiovascular diseases in 2 (3.6%) mothers (χ^2=7.18, p=0.304). Also, there was no association between the occurrence of vaginal delivery or cesarean section birth and the development of necrotizing enterocolitis (χ^2=0.88, p=0.64).

Physical development indicators did not differ statistically between the comparison and main groups, but a statistically significant difference was noted between the control group and both groups. The physical development indicators of the control group children were higher than those of the other two groups (p=0.001). While comparing the physical development indicators of 88 children with suspicon of necrotizing enterocolitis according to the confirmation of diagnosis, a statistical difference was noted only in terms of height. In children with an unconfirmed diagnosis of necrotizing enterocolitis, the height was 38.9±0.5 cm (min 30-max 47), and in children with a confirmed diagnosis of NEC, it was 41.2±0.7 cm (min 34-max 48) (p=0.019).

Although, the children of the control group statistically significantly differed from the other groups in terms of Apgar scale scores in our study (p=0.001), there was no statistically significant difference between the main and comparison groups.

There was no statistical difference between groups according to the gender of premature babies (χ^2=0.038; p=0.981). Confirmation of NEC diagnosis occurred regardless of whether the children were in the early or late neonatal period (χ^2=1.539, p=0.215). The condition of the children was assessed as severe, very severe and preagonal,when suspicion of NEC has occured. Children in very severe condition predominated among children with confirmed diagnosis of NEC (n=6, 66.7%-IA s/g, n=13, 65%-II s/g, χ^2=40.2, p=0.001).

Children who received respiratory therapy were investigated among children with suspicion of NEC.

Children receiving respiratory therapy were investigated among children with suspicion of NEC, and when comparing the groups related to respiratory therapy with CPAP, no statistically significant difference was noted (x^2=0.104, p=0.747). At the same time, no difference was noted between the main and comparison groups regarding respiratory support with ASV (x^2=1.339, p=0.247). CPAP therapy prevailed in group 1A and group 2 B (x^2=9.7, p=0.021.)

We did not determine the relationship between cardiac pathologies - patent foramen ovale and ductus Botalli - and the confirmation of the diagnosis of necrotizing enterocolitis during echocardiography examination. At the same time, such a relationship was not noted in the children who died. As tricuspid insufficiency was more common in echocardiography, we also examined the distribution of this pathology among the subgroups and no relationship was identified (p>0.05).

Considering that NEC would be more common in the main group, a comparison of the main and comparison groups was made according to the confirmation of the NEC diagnosis during the study, but no statistically significant difference was noted (p>0.05).

The presence of occult blood in the stool, which is considered one of the initial clinical signs of NEC, was investigated, but no statistical difference was noted in the presence of occult blood in the stool in children with a confirmed NEC diagnosis compared to the other group (x^2=1.88, p=0.596).

Abdominal ultrasound and X-ray examinations were performed dynamically in children.

According to the results of X-ray examinations, the main and comparison groups did not differ statistically significantly from each other (x^2_{R1}=1.009; p=0.799; x^2_{R2} =1.394; p=0.707).(R_1-initial,R_2- X-ray in dynamics).

During the initial radiological examination, radiological signs were not statistically significant in confirming the diagnosis of necrotizing enterocolitis (x^2=3.377; p=0.337). In dynamics, radiological examination was more statistically significant, the presence of

intestinal wall thickening coincided more with clinical signs, and 7 out of 10 children (70%) with intestinal wall thickening were diagnosed with NEC (x^2=8.476; p=0.037).

In children with clinically confirmed diagnosis of necrotizing enterocolitis, in dynamics, radiologically stage I was noted in 24 cases, stage II in 1 case, and stage III in 3 cases.No radiological changes were noted in one case (82.8%-I, 3.4%-II, 10.3%-III) (χ^2=36.7, p=0.001).

Stage III of necrotizing enterocolitis was observed in 3 children who underwent surgery (χ^2=45, p=0.000). 3 children died due to the development of cardiovascular failure in stage I, and 2 died after surgery performed in stage III (x^2=5.98,p=0.113).

Abdominal ultrasound was performed on the day of suspicion and in dynamics in all children with suspicion of necrotizing enterocolitis. No statistically significant difference was noted in the distribution of ultrasound signs in the groups (x^2=3.72; p=0.875).

The role of US indicators in confirming the diagnosis of NEC is also insignificant (x^2=11.906; p=0.104).

Characteristics of hematological indicators in premature infants with suspicion of necrotizing enterocolitis

In order to investigate the role of hematological indicators, especially anemia, in the development of necrotizing enterocolitis and at the same time, the observed disorders, the complete blood count indicators of infants with suspected NEC were compared with the control group on the first day and in dynamics on the 5-7th day ,as well as in subgroups according to the development of NEC.

Whilst significant statistical differences were noted in comparison with the control group, there was no statistically significant difference between the main and comparison groups. Dynamic indicators did not differ between the main and comparison groups also. While investigating the dynamic changes in the main and comparison groups, especially Hb, Hct, MCV, MCH, RDW-SD decreased statistically significantly in dynamics ($p<0.05$). The number of platelets increased in both the main and comparison groups and was statistically significant ($p<0.05$). Lymphocytes increased in dynamics, especially in the comparison group ($p<0.05$). An increase in PDW was also noted in this group of children ($p<0.05$).

Particularly, in order to investigate the role of hematological indicators in confirming the diagnosis of necrotizing enterocolitis in children suspected of NEC, dynamic changes were studied in subgroups. Erythrocytes, Hb, Hct, MCV, MCH and RDW-SD decreased in dynamics and were statistically significant ($p<0.05$) in subgroup B. Lymphocytes increased in dynamics ($p<0.05$). The same changes also occured statistically significant in subgroup A, at the same time, PDW-platelet distribution width increased in these children ($p<0.05$).

The degrees of anemia were classified based on Hct indicators, and we tried to study their role in the development of NEC during the research work. No difference was noted in the distribution of anemia between subgroups according to the results of both- the initial and dynamic examination ($p>0.05$).

Continuous monitoring of blood gases and acid-base status was carried out in children suspected of NEC. There was no statistically significant difference in blood gases and acid-base status between groups I and II (main and comparison), but when comparing sub-groups, PvO_2 increased especially in subgroup A ($p=0.022$). One of the interesting points in blood gas examination is the statistically significant difference in FO_2Hb in newborns of subgroups I and II A (84.45±6.17 in subgroup IA; 91.8±3.53 in subgroup IIA; $p=0.001$). FHHb was higher in subgroup IA (11.69±6.50 in IA s/g; 4.23±3.58 in IIA s/g; $p=0.008$). In dynamics, $FHHb_2$ increased in subgroup II A children, and was statistically different from subgroup I B children ($p=0.049$).

C-reactive protein was determined at the initial examination and in dynamics, and its role in the early diagnosis of necrotizing enterocolitis was studied by comparing groups and subgroups, and no statistically significant difference was noted between the groups.

A statistical difference was noted in the dynamics of CRP levels between subgroups within group I (CRP level in dynamics IAs/g -13.5; IB s|g -4.46 mg/l) ($p<0.05$).

The levels of Na+, K+, Ca++ and Cl^- ions were studied in all children with suspected necrotizing enterocolitis. No significant difference was noted in the levels of microelements in groups I and II.

The Ca level in dynamics was low in subgroup A (initial 1.13±0.06; in dynamics 1.06±0.06, p=0.04). At the same time, the Ca level differed between the I B subgroup and the II A subgroup (Mean±SD 1.3±0.32 in IB s/g; 1.12±0.37mmol/l in IIAs/g) (p=0.038). The Cl ion level was also lower in children with a confirmed diagnosis of necrotizing enterocolitis (106.7±1.49 in As/g at the initial examination; 111.7±1.13mmol/l in B s/g, p=0.025). In general, the K^+ level was not significant in confirming the diagnosis in children suspected of NEC.

We constructed the ROC curve considering the statistical significance of the difference and the fact that the Ca ion level was lower in children with a confirmed NEC diagnosis in dynamics, than in other children.

The area under the curve was higher for children with an unconfirmed NEC diagnosis and was statistically significant. Area=0.643, standard error (SE)=0.68, Confidence interval (CI) (95%)=0.51-0.76, p=0.043. The cut-off level was 1.25 mmol/L, sensitivity-66.7%, specificity-67%. This shows that in 66.7% of cases, the diagnosis of NEC is not confirmed when the Ca ion level is more than 1.25. The Cl ion level was also low in children with a confirmed diagnosis of necrotizing enterocolitis, (p=0.025), the ROC curve and cut-off level were determined. The area under the ROC curve of Cl ion was=0.651, SE=0.064, CI(95%)=0.525-0.777 and was statistically significance (p=0.025). The cut-off level was 110.5 mmol/l, sensitivity 53.8%, and specificity was 79.3%.

The role of near-infrared spectroscopy in the early diagnosis of necrotizing enterocolitis in premature infants

This chapter is about the role of near-infrared spectroscopy in the diagnosis and prognosis of necrotizing enterocolitis by measuring peripheral intestinal tissue oxygenation with NIRS.

Intestinal tissue oxygen saturation was measured in the infraumbilical region using a pediatric somasensor, and brain tissue saturation was measured in the frontoparietal region, and continuous pulse oximetry was applied to the patient at the same time; brain and splanchnic tissue oxygen saturation (r_cSO_2 and r_sSO_2, respectively) was measured, and the splanchnic cerebral oxygenation ratio- SCOR (widely used to assess perfusion and metabolism of the examined

region: r_sSO_2/r_cSO_2 x100%) and FTOE (Fractional Tissue Oxygen Extraction: SpO_2-rSO_2/SpO_2) were evaluated. NIRS indicators were compared in all children with suspected necrotizing enterocolitis with control group of children.

Comparison of the evidence from the study revealed that r_cSO_2 values were significantly lower in children with suspected NEC compared to the control group (74.13%±10.09; 80.66%±4.53; p=0.000).

cFTOE and sFTOE values were significantly higher in children with suspected of NEC compared to the control group (respectively; cFTOE 0.25±0.11; control group 0.218±0.17; p=0.003 and sFTOE 0.59±0.17; control group 0.222±0.89; p=0.000). SCOR indicators were significantly lower in children with suspected of NEC than in children in the control group (0.53±0.21 and 0.95±0.11, respectively). r_sSO_2 levels were approximately 2 times lower in children with suspected of NEC, but did not differ statistically.

Regarding the NIRS indicators by groups, all measurements of near infrared spectroscopy in children within the control group were statistically significant compared to other groups. There was no difference between the main and comparison groups (p>0.05). It is noted that r_cSO_2 was statistically significantly lower in the comparison group compared to children in the control group (73.29%±10.54; 80.66%±4.53; p=0.000).

While evaluating the cFTOE and sFTOE parameters,significant statistical difference was noted between the comparison and control groups (comparison group 0.270±0.116; control group 0.22±0.17; p=0.001 and comparison group 0.595±0.167; control group 0.23±0.09, p=0.000).

Concerning the SCOR index,was lower in the comparison group than in the control group children (0.55±0.21 and 0.95±0.11, respectively), but it did not differ statistically. The r_sSO_2 level was approximately 2 times lower in the comparison group, but it did not differ statistically significantly (p>0.05).

Oximetry indicators, especially r_cSO_2, were also significantly lower in the main group compared to the control group (p=0.007). cFTOE and sFTOE indicators were higher compared to the control group (p=0.001).

When analyzing the NIRS indicators of all children suspected of NEC according to NEC confirmation, we realize that r_cSO_2 indicators in children with confirmed NEC are statistically significantly lower than those without NEC confirmation (70.83±7.97 in confirmed NEC; 75.76±10.68 in unconfirmed NEC) (p=0.049). cFTOE indicators were higher in children with confirmed necrotizing enterocolitis than those with unconfirmed diagnosis (0.297±0.11 in confirmed NEC; 0.234±0.11 in unconfirmed NEC) (p=0.026).

As for other oximetry indicators, no statistically significant difference was noted between them (p>0.05). Based on the greater statistically significant difference of cFTOE, we constructed a ROC curve in order to clarify its role in confirming the diagnosis of NEC (figure 1). The size of the area under the curve was statistically significant.

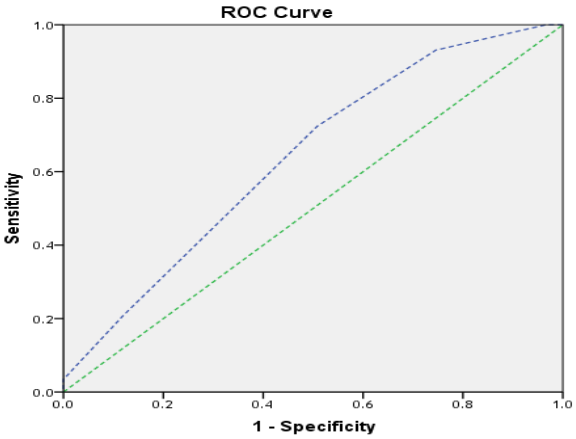

Figure 1. ROC curve of cFTOE in children with confirmed necrotizing enterocolitis

The cut-off level for cFTOE was 0.250; sensitivity was 72% and specificity was 49%. The area under the curve (AUC) for cFTOE was 0.639 (p=0.035). The higher AUC result for cFTOE indicates that this significative has a higher prognostic power.

Several studies have shown the diagnostic and prognostic significance of r_sSO_2 variability in the diagnosis of NEC. We also tried to study the variability by performing NIRS measurement for 2 hours (r_sCoVAR=SD/Mean) in our study.

Abdominal variability is lower in premature infants with diagnosis of confirmed NEC, than in those without NEC, and a statistically significant difference is noted ($p=0.043$; $p<0.05$).

We also compared NIRS indicators depending on gestational age in children examined by us. Thus, gestational age was divided into 4 groups: <28 weeks, 28-30 weeks, 31-33 weeks and 34-36 weeks, and NIRS indicators were compared in these groups. Both abdominal and cerebral oximetry indicators changed depending on gestational age. So, as gestational age increased, r_cSO_2 and r_sSO_2 indicators are increased. When comparing groups (I-III, II-IV), we witness that oxygen transport to visceral tissues - r_sSO_2 - increased by 2 times.

In contrast, FTOE indicators decrease with the rising of gestational age. The SCOR index also tended to increase with increasing gestational age, especially in group IV ($p<0.05$).

r_cSO_2 was higher in group IV, while cerebral oximetry was lower in group I compared to other groups, which was statistically significant compared to group IV ($p<0.05$). Abdominal oximetry indicators were statistically significantly lower in group I than in other groups ($p<0.05$). At the same time, the sFTOE indicator was significantly differed between group I, III and IV ($p<0.05$). The SCOR index was statistically significantly different in all groups ($p<0.05$). Also, the correlations of NIRS indicators with physical development signs and gestational age were investigated in all children.

When focusing on the correlations between gestational age and NIRS indicators, a strong positive (direct) correlation relationship with abdominal and cerebral oximetry indicators ($p<0.01$) and a strong direct correlation with SCOR indicator ($p<0.01$) was noted in all children. However, an inverse correlation relationship was noted between both abdominal and cerebral FTOE indicators and gestational age ($p<0.01$). The same correlation relationships were noted between body weight and NIRS indicators. A direct correlation relationship was noted between body weight and abdominal and cerebral oximetry

indicators, also SCOR indicators (p=0.001) and an inverse correlation relationship was noted with both cerebral (p=0.003) and abdominal FTOE indicators (p=0.001).

When it comes to height figures, we witness the same situation being noted here (p<0.01).

More correlations were noted between blood parameters and r_cSO_2. Thus, a direct correlation was noted between r_cSO_2 and RBC, Hb, Hct (r=0.360,p=0.001; r=0.412,p=0.001; r=0.365, p=0.001, respectively), and an inverse correlation was noted with PDW (r=-0.314, p=0.019).

Also, an inverse correlation in dynamics (r=-0.322, p=0.005) was noted between CRP and r_cSO_2.

The role of claudin-3 protein in early diagnosis of necrotizing enterocolitis in premature infants

The comparison of claudin-3 levels in examination groups was studied in our research work.

In total, claudin-3 levels were determined in urine of 69 children. Among them, 45 children were suspected of necrotizing enterocolitis, and 24 children were in the control group. In 30 cases of 45 children, the diagnosis of NEC was not confirmed, and in 15 cases, it was confirmed.

In conditionally healthy premature children, the level of claudin (Mean±SD) was 10.90±1.63ng/ml, min 7.90, and max 13.80 ng/ml, which was 3 times lower than in other groups. In the main group (Mean±SD) it was 30.28±6.72ng/ml, min 22.10, and max 48.80ng/ml, and in the comparison group (Mean±SD) it was 31.39±4.83ng/ml, min 22.60, and max 39.60ng/ml, and the statistical significance of the difference was (p<0.001).

Notwithstanding a statistically significant difference between all three groups (control, confirmed NEC, and unconfirmed NEC), claudin-3 values were more than 3-fold higher in children with confirmed NEC compared to the control group (34.03±4.20 ng/ml; 10.9±1.63 ng/ml, respectively) (p<0.05). Because of a strong statistical difference between the groups, we constructed a ROC curve to find the sensitivity and specificity of the significance of claudin-3 protein in confirming the diagnosis of NEC (figure 2).

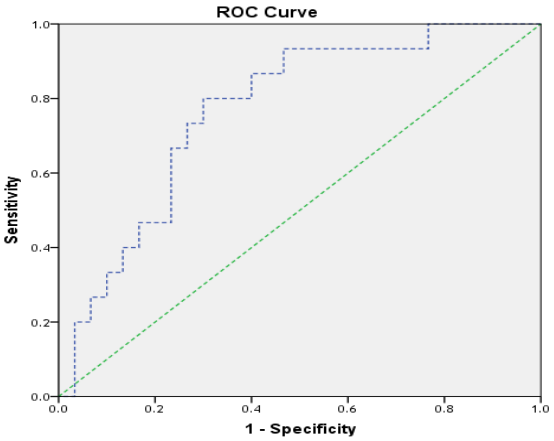

Figure 2. Claudin -3 ROC curve.

The higher result of area under the curve (AUC) (0.769) indicates that claudin has a high diagnostic value. We determined the diagnostic cut -off level by finding the indicator that is farthest from the midline based on the sensitivity and specificity indicators. A claudin-3 level higher than 31.5 ng/ml confirmed the diagnosis of NEC with 80% sensitivity. A claudin-3 level lower than 31.5 ng/ml could deny the diagnosis with 70% specificity.

Correlations between claudin-3 levels and NIRS indicators, also hematological indicators were investigated.

There were inverse correlations between body weight, height, gestational age and claudin-3 (r=-0.647,r=-0.654,r=-0.628,p=0.001, respectively). The presence of a direct correlation between cFTOE and sFTOE (r=0.288, p=0.016; r=0.636, p=0.001, respectively) of NIRS indicators reflects the increase in oxygen extraction and the greater incidence of intestinal damage in hypoxic conditions. Considering that SCOR indicators are a marker of intestinal perfusion disorders, it is comprehensible that claudin-3 indicators increase when this indicator decreases (r=-0.602, p=0.001). A strong inverse correlation relationship was noted between Hb, Hct and RBC (r=-0.537, r=-0.484, r=-0.490, p=0.001, respectively) among blood indicators.

Since a strong correlation was noted between body weight indicators, SCOR and claudin-3 levels ,a linear regression relationship was investigated in children with suspected of NEC. A model was developed based on the formula y=a+b1 X x1+b2 X x2 ($p<0.001$):

Claudin-3=49-(0,011 x body weight)-(11,2 x SCOR)

Body weight was more significant with a standardized coefficient of 0.504, and NIRS indicator had a standardized coefficient of 0.281. The R^2 of the model was 0.531, from which we calculate the Cohen effect size. Cohens f=$\sqrt{}$ 0.531/(1-0.531)= $\sqrt{}$ 1.132=1.06. The number of Cohens higher than 0.4 indicates a high effect size, reflecting the importance of body weight and SCOR for claudin levels.

One of the goals of our study was to predict the outcome of NEC. While studying the prognostic significance of our markers in case of death, lactate levels were of particular interest.

Both , the initial and dynamic levels of lactate were significantly higher in children who died. Initial Lac (Mean±SD) in dead infants was 5.2±4.5mmol/l, min 0.9, max 20, and initial Lac in the survivors was 3.2±1.9 min 0.6, max 10.5; p=0.005. In dynamics, Lac was 9.7±8.5 min 1.6, max 27 in dead infants, and 2.6±1.8, min 0.6, max 13.50 in survivors (p=0.001).

We performed ROC analysis to predict mortality based on the initial Lac level in children with suspected of NEC. The area under the ROC curve (AUC) was 0.672, p=0.037; $p<0.05$, (area±SE) was 0.672±0.08. The cut-off level was set at 3.45 mmol/l, with a sensitivity of 73%; specificity of 59%.

Among the NIRS indicators, r_cSO_2 was statistically significant between the dead infants and survivors (68.2±8.8 in dead children and 75.4±9.9 in survivors; p=0.012). r_sSO_2 was significantly lower, but not statistically different (p=0.78).

Claudin-3 levels were not statistically significant in predicting mortal outcome ($p>0.05$).

Figure 3 shows the diagnostic and prognostic algorithm for NEC. According to the algorithm, it starts with suspicion of NEC and the presence and course of NEC are predicted using various tests and criteria in the diagram stages.

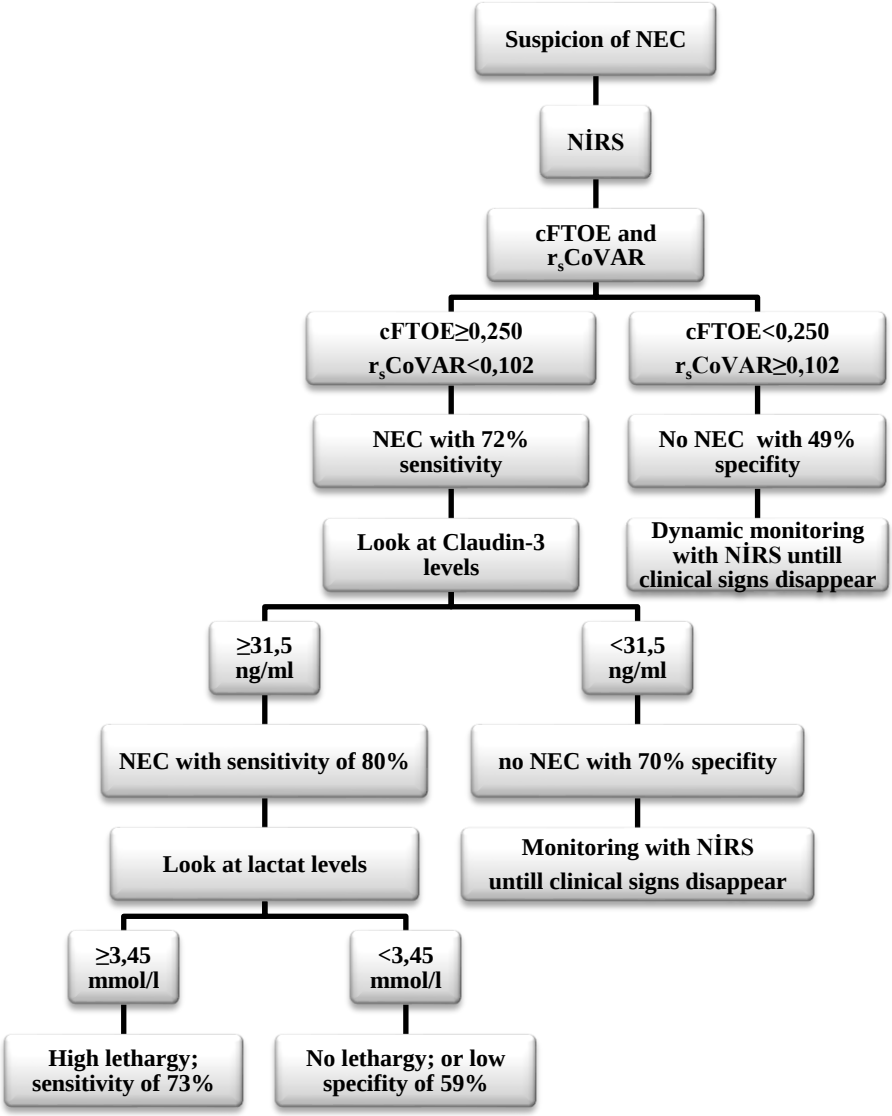

Figure 3. Diagnostic and prognostic algorithm of NEC

Firstly, the patient with suspected of NEC is evaluated by the NIRS (Near-Infrared Spectroscopy) examination method.

cFTOE and r_sCoVAR values are examined and cFTOE and

r_sCoVAR are evaluated. cFTOE≥0.250 and r_sCoVAR<0.102: indicates the presence of NEC with a sensitivity of 72%, cFTOE <0.250 and r_sCoVAR ≥0.102 values confirm the absence of necrotizing enterocolitis. If NEC is present according to NIRS indicators, then claudin-3 levels should be examined: NEC is present with a sensitivity of 80% at a claudin-3 value ≥31.5 ng/ml; claudin-3 <31.5 indicates the absence of NEC, and lactate levels should be examined if claudin-3 ≥31.5 ng/ml. Lactate levels ≥3.45 mmol/l predicts mortality with a sensitivity of 73%; lactate <3.45 predicts a favorable outcome. If claudin-3 <31.5, the patient is monitored with NIRS until clinical signs subside.

The presented model is an algorithm designed for the diagnosis and prognosis of patients with suspected NEC.

RESULTS

1. Mothers of infants with confirmed NEC diagnosis had a history of complicated obstetrics twice as often (33.3% and 16.4%, respectively; x^2=3.04, p=0.08) and were, with the infants predominantly being born from the third and later pregnancies (x2=20.7, p=0.004). [15].

2. The diagnosis of necrotizing enterocolitis was established in 82.8% of cases at stage I, 3.40% at stage II, and 10.3% at stage III of the disease [16].

3. Cerebral oximetry NİRS indicators showed a significant decrease in r_cSO_2 (p<0.05); while cFTOE was higher (p<0.05). Among abdominal oximetry parameters, a marked reduction in abdominal variability (r_sCoVAR) was of early diagnostic importance (p<0.05) [7,14].

4. High urinary claudin-3 levels corresponded with NEC development in premature infants suspected of necrotizing enterocolitis (Mean±SD 34.03±4.20 ng/ml, min 24.5, max 41.20 ng/ml in those with confirmed NEC; 29.3±5.82 ng/ml, min 22.1, max 48.8 ng/ml in those with unconfirmed cases of necrotizing enterocolitis; and in control group of infants it was 10.9±1.63 ng/ml, min 7.90, max 13.80,compared among all groups p<0,05) [3,6].

5. A strong direct correlation (p=0.001) was observed between claudin-3 levels-indicating intestinal barrier disfunction- and the cFTOE and sFTOE NIRS indicators, an inverse correlation (p=0.001) was found with the SCOR index [5].

6. A diagnostic and prognostic algorithm was developed based on our results. Urinary claudin-3 levels ≥31.5ng/ml (80% sensitivity, 70% specificity, $p<0.05$), NIRS cFTOE≥0.250 (72% sensitivity, 49% specificity), and splanchnic variability (r_sCoVAR)<0.102 were identified as diagnostic and prognostic criteria of necrotizing enterocolitis, and initial lactate levels higher than 3.45mmol/l (73% sensitivity and 59% specificity) were evaluated as prognostic marker for an unsatisfactory outcome in premature infants [6,7,14,15,16].

PRACTICAL RECOMMENDATIONS

1. It is recommended to use a non-invasive, easy-to-perform near-infrared spectroscopy examination for the early diagnosis and prognosis of necrotizing enterocolitis,for determining the strategies of examination and treatment in practical experience in premature infants.

2. A urinary claudin-3 level ≥31.5ng/ml is recommended for the early diagnosis of necrotizing enterocolitis, and an initial blood lactate level higher than 3.45 mmol/l is recommended for predicting an unsatisfactory outcome of the disease in premature infants.

3. It is recommended to use the diagnostic and prognostic algorithm we developed for assessing necrotizing enterocolitis in premature infants to facilitate the work of neonatologists in practice.

The list of published scientific works on the dissertation topic

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ABBREVIATIONS

CPAP	–	continuous positive airway pressure
FTOE	–	fractional tissue oxygen extraction
MV	–	mechanical ventilation
NEC	–	necrotizing enterocolitis
NIRS	–	Near Infrared spectroscopy
PI	–	premature infant
r_cSO_2	–	cerebral regional tissue oxygen saturation
r_sSO_2	–	splanchnic regional tissue oxygen saturation
r_sCoVAR	–	Abdominal variability
RDS	–	Respiratory distress syndrome
ROC	–	Receiver operating characteristic (statistic method)
SCOR	–	splanchnic-cerebral oxygenation ratio
US	–	ultrasound
WHO	–	World Health Organization

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