Serum Neopterin Concentration in Children with Viral Gastroenteritis

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Abstract

Increased neopterin concentrations are observed in many diseases in which cellular immunity is stimulated. Gastroenteritis is still a major cause of childhood morbidity and mortality worldwide. Over 75% of all cases are caused by viral infections. Therefore a non-specific, quick, sensitive, cheap and easy-to-perform marker of viral infection, such as neopterin concentration in serum, would be a cost-effective method of identifying patients with gastroenteritis into probable viral etiology and therefore it would optimize treatment and reduce inappropriate antibiotic treatment.

This study evaluated whether serum neopterin concentration can be a reliable marker of viral etiology of gastroenteritis in children. The study group consisted of 67 children: 29 girls and 38 boys, aged from 0.1 to 16.1 years with symptoms of gastroenteritis and with confirmed rotavirus or adenovirus infection; the control group consisted of 105 healthy children. Clinical examinations and laboratory test including whole blood count, serum C-reactive protein, procalcitonin, and creatinine concentration were conducted. The serum neopterin level was analyzed by Elisa.

Serum neopterin concentration in the study group varied from 6.03 to 171 nmol/L (median 23.7 nmol/L) and it was significantly higher than in the control group (from 2.88 to 14.8 nmol/L; median 4.73 nmol/L) (P < 0.001). Neopterin concentration was above the normal value (>11 nmol/L) in 58 children from the study group and only in 6 from the control group (86.6% sensitivity and 94.3% specificity). Serum neopterin concentration did not differ significantly between children with rotavirus and adenovirus infection (P = 0.47). The correlation between serum neopterin and C-reactive protein concentrations was of borderline significance (r_s = 0.23, p = 0.06); and there was no correlation between serum concentrations of neopterin and procalcitonin, white blood count, lymphocytes count, monocytes count or the body temperature.

Serum neopterin concentration is highly increased in children suffering from viral gastroenteritis, regardless of the virus type. When a cut-off of 11.0 nmol/L neopterin was applied, comparison yields 86.6% sensitivity and 94.3% specificity for the diagnosis of viral gastroenteritis in pediatric patients. Further studies are necessary for other etiological agents of gastroenteritis.

Key words: neopterin, C-reactive protein, procalcitonin, creatinine, gastroenteritis, rotavirus, adenovirus

Introduction

Pteridine derivative neopterin is released by activated monocytes and macrophages under the stimulation of interferon gamma which is secreted by active Th1-type lymphocytes (1). Therefore neopterin has been described as a biochemical indicator of cell-mediated immune response (2). Its concentration has already been evaluated in serum, urine, cerebrospinal fluid, and in articular fluid. Cell-mediated immune response is activated in infections, autoimmune diseases, malignant tumor diseases, in some neurological and cardiovascular diseases, and in allograft rejection episodes (3, 4).

Since the first observation of increased neopterin secretion in urine of patients with viral infections by Wachter et al. (5), a great number of researches concerning elevation of neopterin concentration in body fluids as a parameter differentiating viral from bacterial infections have been made (4, 6, 7). High neopterin concentration has been observed in HIV infections (8), acute hepatitis (9), rubella (10), several herpes infec-
Gastroenteritis is still a major cause of childhood morbidity and mortality worldwide. Over 75% of all cases of gastroenteritis are caused by viral infections; rotavirus, adenovirus, human calcivirus, and astrovirus are the most common etiological agents. Rotavirus alone is responsible for 20-60% of cases of gastroenteritis requiring hospitalization (14). Each year, rotavirus causes approximately 111 million episodes of gastroenteritis and 352,000-592,000 deaths in children <5 years of age. By the age of 5 years, nearly every child will have an episode of viral gastroenteritis, 1 in 5 will visit a clinic, 1 in 65 will be hospitalized, and approximately 1 in 293 will die. Children in the poorest countries account for 82% of rotavirus deaths. These data were estimated on the analysis by Parashar et al., of all the studies published during fourteen years on deaths caused by diarrhea and on rotavirus infections in children (15).

Techniques for the detection of specific viral antigens in fecal samples are based on EIA, agglutination with latex particles or immunochromatography. Although all of these tests are commercially available for group A rotavirus, adenovirus and astrovirus, in everyday practice, due to relatively high costs, only one or two tests are usually performed and only in specialized pediatric centers (14). Exclusion of bacterial infection by stool cultures is also expensive and, moreover, time-consuming. Therefore a non-specific, quick, sensitive, cheap and easy-to-perform marker of viral infection, such as neopterin concentration in serum or C-reactive protein concentration to neopterin ratio, would be a cost-effective method of identifying patients with gastroenteritis into probable viral etiology and therefore it would optimize treatment and reduce inappropriate antibiotic treatment.

The aim of the present study is to evaluate whether serum neopterin concentration can be a reliable marker of viral etiology of gastroenteritis in children.

Materials and Methods

The studied group consisted of 67 children: 29 girls (43.3%) and 38 boys (56.7%), aged from 0.1 to 16.1 years (mean 6.2 ± 4.2 years, median 6.6 years) with clinical symptoms of gastroenteritis. In all the patients viral etiology of the disease has been proved by rapid test for detecting rotavirus and adenovirus antigens in stools using immunochromatographic technique (VIKIA Rota-Adeno, BioMerieux, France). Other causes of gastroenteritis were excluded by detailed history, careful clinical examination, stool cultures, and, if necessary, urine or and blood cultures, otoscopic examination, or chest X-ray were performed to eliminate secondary gastrointestinal disturbances.

The control group consisted of 105 healthy children: 47 girls (44.8%) and 58 (55.2%) boys, aged from 0.1 to 17.9 years (mean 7.6 ± 5.7 years, median 7.2 years). All of the children were admitted to hospital for a surgery due to a correction of different congenital malformations.

In all children the detailed history was taken and careful clinical examinations and laboratory test were performed. Patients with any chronic infection, autoimmune or neoplastic disease, and the ones who had been vaccinated or had received the antibiotics therapy during the previous 4 weeks were excluded from the study. Children with body weight below third percentile for height did not enter the study either, as protein-caloric malnutrition may compromise cellular immunity. None of the children was treated with steroids, immunosuppressive or immunomodulatory therapy during the 3 months before the study.

Evaluation of neopterin concentration in serum

The blood samples were carefully protected from sunlight, then in less than 20 minutes after collection they were centrifuged at 3,000 rpm for 10 minutes. The obtained serum was frozen and stored at -20°C. Measurement of serum neopterin concentration was performed using a commercial immunoenzyme assay (ELISA) for quantitative measurement of serum neopterin concentrations (Brahms Corporation, Hennigsdorf, Germany). This test uses technique of antibody-covered plates.

Other performed laboratory tests included
Whole blood count with white blood count - analyzed by standard method.

Serum C-reactive protein concentration (CRP), established by immunoturbidimetric method with latex test.

Serum procalcitonin concentration (PCT), established by quantitative immunoluminometric method using commercial LIA PCT kit by BRAHMS Corporation, Hennigsdorf/Berlin, Germany.

Serum urea (by urease method with GLDH) and creatinine concentration (by Jaffe’s method) - established to confirm the normal kidneys function.

Statistical analysis
The results of the study were analyzed statistically using Statistica 8 (StatSoft, Inc., Tulsa, OK, USA). The correlation of parameters was analyzed by Spearman rank test and differences among groups of unpaired data by distribution-free Mann-Whitney test.

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A P-value of <0.05 was regarded as statistically significant.

All tests were performed in children with written consent of their parents/legal tutors who were informed about the aim and assumptions of the study. The consent of Independent Bioethical Committee for Scientific Research of the Medical University of Gdansk was obtained to perform these studies (NKEBN/942/2004).

Results

In the study group rotavirus infection was diagnosed in 60 cases (89.6%) and adenovirus in 7 (10.4%). Urea and creatinine concentrations were within the normal for age values in all the children.

Serum neopterin concentration in the study group ranged from 6.0 to 171 nmol/L (mean 33.4 ± 30.6 nmol/L, median 23.7 nmol/L) and it was significantly higher than in the control group (from 2.88 to 14.8 nmol/L; mean 5.47 ± 2.58 nmol/L, median 4.73 nmol/L) (P <0.001) as it is shown in Figure 1.

Serum neopterin concentration did not differ significantly between children with rotavirus and adenovirus infection (not significant) (Figure 2) and in both subgroups was significantly higher than in the control group (P <0.001).

Reference standard of serum neopterin concentration in the control group has been previously evaluated by mean value ± 2*standard deviation (mean ± 2SD) methods. Based on this rule the upper limit value of reference standard of serum neopterin level (cut-off value) was established as 11 nmol/L for healthy children older than 1 month, independently of their age and gender (16).

In the study group of children with viral gastroenteritis serum neopterin concentration was above the normal value in 58 (86.6 %) patients and was normal in 9 (13.4 %) patients. Only 6 (5.7 %) children out of 105 from the control group had serum neopterin level above the upper limit value. These data provide 86.6 % sensitivity and 94.3 % specificity of elevated serum neopterin concentration in children with gastroenteritis with positive predictive value 0.906 (90.6%) and negative predictive value 0.917 (91.7%).

Serum CRP was above the normal value (>5.5 mg/L) in 12 children with gastroenteritis (17.9 %) and in 8 children from the control group (7.6 %). That means 17.9 % sensitivity and 92.4 % specificity of elevated serum CRP concentration in children with gastroenteritis.

Serum procalcitonin level was not significantly elevated (<2.0 ng/mL) in all the patients from the study group and from the control group.

White blood count exceeded normal-for-age values in 4 children with gastroenteritis (6.0 %) and in 3 children from the control group (2.9 %). These data provide 6.0% sensitivity and 96.2 % specificity of elevated white blood count in children with gastroenteritis.

In the study group there were no correlations between serum neopterin concentration and: serum CRP concentration (rs = 0.23, P = 0.06) (Figure 3); serum procalcitonin concentration (rs = 0.03, P = 0.81) (Figure 3); or white blood count (rs = -0.08, P = 0.53) (Figure 3).

In the study group there were also no correlations between serum neopterin concentration and: lymphocytes count (rs = 0.05), monocytes count (rs = 0.17), or the body temperature (rs = 0.20, all p-values not sig-
CRP (mg/L) to neopterin (nmol/L) concentration ratio (CRP/NPT) was evaluated in both the study and the control group. In the study group CRP/NPT ratio varied from 0.02 to 2.23 mg/nmol, median 0.15 mg/nmol while in the control group varied from 0.03 to 1.96 mg/nmol, median 0.31 mg/nmol, P<0.001. CRP/NPT ratio was lower than 1.0 mg/nmol in 63 children with viral diarrhea (94.0%) and in 97 healthy children (92.4%). Sensitivity of CRP/NPT ratio values below 1.0 mg/nmol was 92.4% and specificity 7.6%. CRP/NPT ratio below 3.0 mg/nmol was observed in all the patients, both from the study and from the control group.

**Discussion**

Clinical usefulness of evaluation of serum neopterin concentration has been analyzed in many different fields of medicine but probably most widely in infectious diseases. High neopterin concentration in body fluids is observed most of all in infections of viral etiology while during bacterial infection, excluding some intracellularly proliferating bacteria, its concentration is usually low. There are several biochemical markers of bacterial infections which are routinely measured in clinical practice, like C-reactive protein and other acute-phase proteins (e.g. α 1-antitrypsin, α 2-macroglobulin, fibrinogen, ferritin, haptoglobin, serum amyloid, etc.), procalcitonin concentration in serum or ESR but specific parameters for viral infections are lacking. Only neopterin concentration in serum or in other body fluids seems to be of clinical value as such an indicator.

Epidemiological data indicate that viral infections are the most frequent cause of gastroenteritis in children worldwide and the prevalence of rotavirus infections is evident (14). Our study focuses exclusively on serum neopterin concentration in children suffering from viral gastroenteritis. Extremely high values, reaching 171 nmol/L, were obtained in the study group and median value of neopterin was over 5 times higher than in the control group. Estimation of serum neopterin concentration exceeding the normal values for children (>11.0 nmol/L) gave very high sensitivity (86.6%) and specificity (94.3%) for the diagnosis of viral gastroenteritis.

Similar data were presented by Denz et al.: high concentrations of serum neopterin concentration were observed in patients suffering from acute viral infection (respiratory tract infections, type A hepatitis and diarrhea) while normal or only slightly increased values were obtained in patients with acute bacterial infection (pneumonia) (6).

Okumara et al. performed the comparison of serum neopterin concentration in 17 children infected with influenza virus type A with the similar in number
A study of urine neopterin concentration in children with infections of different etiology by Niederwieser et al. showed increased neopterin concentration in 14% of patients with bacterial infections (pneumonia, meningitis) and in over 90% of children with viral infections (chickenpox, mumps, viral pharyngitis) although the authors underlined that the estimation of neopterin concentration was a non-specific parameter and it allowed only to evaluate the activation of cellular immune response (18).

Urine neopterin concentration in children with different diseases was also studied by Graessl et al. In all the children with infectious diseases (among them 29 with rotavirus infection, 9 with other viral infections, and 5 with bacterial infections) the urine neopterin concentration was significantly higher than in healthy children and than in children suffering from bronchial asthma (13).

In the present study, the comparable serum neopterin concentration in children infected with rotavirus and adenovirus allows assuming that neopterin concentration may also be high in gastroenteritis caused by other, less frequently occurring types of viruses. Performance of multiple tests for many different viral antigens in stools is expensive and routinely impossible in clinical practice. Therefore serum neopterin concentration as a cheap, non-specific test may reduce costs of differential diagnosis in children with gastroenteritis. Whether serum neopterin concentration alone may be used for differentiation between viral and bacterial causes of gastroenteritis will be the point of our further study. Unlike the most of bacterial pathogens, some intracellularly-proliferating bacteria, e.g. known gastrointestinal tract pathogen Salmonella, may induce cell-mediated immune response and therefore stimulate neopterin secretion. In such cases high neopterin concentration might not rule out bacterial infections.

Such conclusions were made by Kozlowska-Murawska and Obuchowicz in the recently published study on acute diarrhea in children. High neopterin concentration was observed in both rotavirus infections and salmonellosis and therefore it could not be helpful in differential diagnosis. Elevated serum CRP concentration indicated bacterial etiology of diarrhea (19). In these specific cases of bacterial infections like intracellularly-proliferating Salmonella, combination of two markers: neopterin and CRP or CRP to neopterin ratio seems to be most valuable. A comparison of serum neopterin concentration in salmonellosis and in other common bacterial diarrhea (e.g. enteropathogenic Escherichia coli) should be the point of further interests.

Similar observations were made by Shaw during analysis of serum neopterin and CRP concentrations in patients suffering from infections of different bacterial (Pneumococcus, Staphylococcus, Streptococcus, E. coli, Klebsiella, Pseudomonas, Salmonella, Meningococcus) and viral (Hepatitis A, CMV, EBV, Rubeola, Paraovirus) origin. The author stated that estimation of neopterin concentration might not serve as a differentiating parameter for these bacterial or viral diseases. Shaw claimed that only CRP (mg/L) to neopterin (nmol/L) concentration ratio might be helpful in differential diagnosis. According to this author, CRP to neopterin concentration ratio above 1.0 mg/nmol indicated bacterial infection while ratio below 1.0 mg/nmol was characteristic for infections of viral origin (7).

Similar observations were presented by Myara et al. (20). In our study, CRP to neopterin concentration ratio below 1.0 mg/nmol occurred to be highly sensitive for differentiating between the group of children with viral gastroenteritis and the control group but the specificity of this ratio was very low.

In the recent study performed by Rainer et al., the roles of neopterin, CRP, and the CRP to neopterin ratio to differentiate bacterial from viral etiology in patients with suspected acute respiratory tract infections were investigated. The median of the CRP to neopterin ratio in patients with bacterial infections was 12.5 mg/nmol, it was 10 times higher than in patients with viral infections (median 1.2 mg/nmol) and 42 times higher than in healthy subjects (0.3 mg/nmol). A cut-off value of CRP to neopterin ratio >3 mg/nmol for ruling in/out bacterial/viral infection yielded optimal sensitivity and specificity for this estimation (21). In our present study CRP to neopterin ratio below 3 mg/nmol was observed in all the children with viral gastroenteritis and in all the children from the control group with median values 0.15 mg/nmol and 0.31 mg/nmol, respectively.

In conclusion, serum neopterin concentration is highly increased in children suffering from viral gastroenteritis, regardless of the virus type. Estimation of serum neopterin concentration exceeding the normal values (>11.0 nmol/L) yields 86.6% sensitivity and 94.3% specificity for the diagnosis of viral gastroenteritis in pediatric patients. Further studies are necessary for other etiological agents of gastroenteritis.
References


