DERLEME/REVIEW ARTICLE

Gülhane Tıp Derg 2013; 55: 237-243

doi:10.5455/gulhane.39000

The indicator of cellular immune response in body fluids: Neopterin

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ABSTRACT

Cytokine levels in body fluids are generally used for evaluation of the immun system's responses to stimuli. But more stable markers to monitoring the inflammatory reactions are needed due to being the majority of cytokines biologically labile, cleaning of the circulation in a short period after secretion quickly, in most cases, acting locally. The measurement of neopterin levels in body fluids could be used to following the activities of cellular immune system due to the stability of molecular structure. Neopterin is a pteridine molecule which 253 dalton molecular weight. Neopterin is synthesized by guanosine triphosphate cyclohydrolase-1 (GTPCH-I) from GTP in body. Neopterin synthesis and secretion increase as a result of stimulation of the cellular immune system. It is understood that, the measurement of the neopterin levels could be helpful to follow-up for cancer patients, the patients undergoing organ transplantation, infectios diseases and clinical conditions which cellular immune system is active such as preeclampsia.

Key words: *Neopterin, Pteridine, Cellular immun system*

ÖZET

Hücresel immun cevabın vücut sıvılarındaki belirteci: Neopterin

Bağışıklık sisteminin uyaranlara verdiği tepkilerin değerlendirilmesinde, genellikle vücut sıvılarındaki sitokin düzeyleri kullanılmaktadır. Ancak sitokinlerin büyük çoğunluğunun biyolojik olarak labil olması, salınımdan kısa bir süre sonra dolaşımdan hızla temizlenmesi ve çoğu durumda lokal etkiler göstermesi gibi nedenlerle, inflamatuar reaksiyonların monitörize edilmesinde daha stabil belirteçlere ihtiyaç duyulmaktadır. Moleküler yapısının stabil olması nedeniyle vücut sıvılarında neopterin düzeylerinin ölçümünün, hücresel bağışıklık sisteminin aktivitelerini takip etmek için kullanılabileceği düşünülmektedir. Neopterin, pteridin yapısında yaklaşık 253 dalton ağırlığı olan bir moleküldür. Neopterin, vücutta guanozin trifosfat siklohidrolaz-I (GTPSH-1) enzimi aracılığıyla GTP'den sentezlenmektedir. Hücresel bağışıklık sisteminin uyarılması sonucunda neopterin sentezi ve salınımı artmaktadır. Yapılan çalışmalar neticesinde; kanser hastalarının, organ transplantasyonu yapılan hastaların, enfeksiyon hastalıklarının, preeklampsi gibi hücresel bağışıklık sisteminin aktif rolü olan klinik durumların takibinde, neopterin düzeylerinin ölçülmesinin faydalı bilgiler verebileceği anlaşılmıştır.

Anahtar kelimeler: *Neopterin, Pteridin, Hücresel bağışıklık sistemi*

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Date submitted: Jun 16, 2013 **Date accepted:** Jun 28, 2013 **Online publication date:** September 26, 2013

Introduction

Neopterin is a molecule which contains pteridine ring in body fluids and tissues and many clinical and experimental studies have been carried on it. In the 1980s, showing urinary neopterin levels increased (1) in patients with malignancy and viral infection, has led the concentration of attention on this molecule. In fact, the history of the studies on pteridines extends to the late 1800s. Pteridines which have been defined as pigments of insects and subphylum vertebrata previously have become the spotlight of attention by the time after understanding it has been a cofactor in tetrahydrobiopterin (H_4B) hydroxylation reactions and the detection of congenital defects in (H_4B) metabolism may lead to hyperphenylalaninemia. Pterins are a group of the compounds known as pteridine. Pteridine structure is obtained by connecting amino group to the second carbon and oxo group to the fourth carbon of ptredine ring. Main pretins in body fluids and tissues are neopterin, ksantopterin, isoxantopterin, pterin-6-carboxylic acid, 6-hydroxymethylpterin and H_4B.

History of Neopterine

Neopterin molecule is found at first in larvae of bees in 1963, later it has been also determined in human urine in 1967 (2). Researches on neopterin have been accelerated (1) by finding high concentrations of nepterin in the urine of children with atypical phenylketonuria (3) and determination of neopterin levels' risings in the urine of patients with malignancy and viral infection. At first the rise of urinary neopterin levels in malignant diseases has been interpreted as cell growth and proliferation have increased the synthesis and release of nepterin and this interpretation has been supported with in

vitro experiments showing the increase of synthesis of some pteridines along with the proliferation of some micro-organisms (1,4-6). However contrary to this hypothesis, later, normal/low neopterin levels were obtained in studies with benign tumors. These findings have suggested that the growth/proliferation of malignant cells or virus-infected cells does not lead the increase of neopterin levels; instead it has suggested the idea of the real responsible mechanism for high levels of neopterin could be related to the immune response of the host (7). The first studies to test this hypothesis have been carried out by the stimulation of peripheral blood lymphocytes enriched in vitro medium with mitogens and allogens. Later it is reported that the urinary neopterin levels of the patients taken under organ/tissue transplant operation has increased after transplant period and this increase has shown correlation with transplant rejection (8). In the early 1980s, in the supernatants of the cultured human peripheral blood mononuclear cells stimulated with antigenic stimuli have been demonstrated accumulation of neopterin (1), and in the light of these datum obtained from all these studies, it is clearly understood that interferon-γ has caused neopterin production and release of large quantities in human macrophages in vitro (5). Thus, it is understood that the elevation of neopterin levels both in in-vitro and in-vivo medium has been occurred through activation of the cellular immune system (8). After this date, studies that suggests neopterin could be considered as a potential marker with a number of clinical and experimental researches have been reported and many in-vitro and in-vivo experiments/researches which shows neopterin biosynthesis is closely related to the activation of the cellular immune system have been done (4, 6). T-lymphocytes releases a number of lymphokines when they have faced foreign substances with antigenic structure. Interferon-γ, one of these lymphokines, stimulates the synthesis and release of neopterin from macrophages. Interferon-γ, mainly produced by activated T-lymphocytes, is the main stimulus for neopterin synthesis. As to the diseases that suppress the immune system or immunosuppressive agents reduce the synthesis of neopterin since they suppress the release of cytokines from T-lymphocytes. Neopterin levels rapidly change in the presence of immunological stimuli. Neopterin levels increase in people with inflammatory disease has been detected more quickly than erythrocyte sedimentation rate or cytokine levels (9). Since this feature enables monitoring the response of immune system more effectively, it makes neopterin a popular molecule.

General Features of Neopterin

Neopterin is in 2-amino-4-oxo-6 (1,2,3-trihydroxy-propyl) pteridine structure and its molecular weight is about 253 daltons. As chemical structure, it has four isomers: D-erythro, L-erythro, D-threo and L-threo (10). Neopterin is synthesized by GTP through guanosine triphosphate cyclohydrolase-I (GTPCH-1) enzyme in the body. GTPCH-1 is an enzyme that has key role in biosynthesis of the pteridines. GTP first transforms into 7,8-dihydroneopterin triphosphate which is an intermediate product and neopterin or dihidroneopterin generate following the hydrolisation by phosphatases. Chemical structure and synthesis steps of neopterin have been shown in Figure 1.

While the highest amount of neopterin in body fluids has been detected in urine, it has a lower level in plasma, CSF and other fluids (11). Neopterin levels in serum samples of healthy people are average 5.65 ± 3.00 nmol/L (12-13). It is reported that neopterin concentrations in healthy people are independent of sex, but some age-related changes have occured (13).

While neopterin emits strong blue fluorescence radiation in neutral or alkaline solutions, it emits the weak fluorescence radiation in acidic solutions (14). Taking advantage of this feature, neopterin measure can be performed through fluorescence detectors

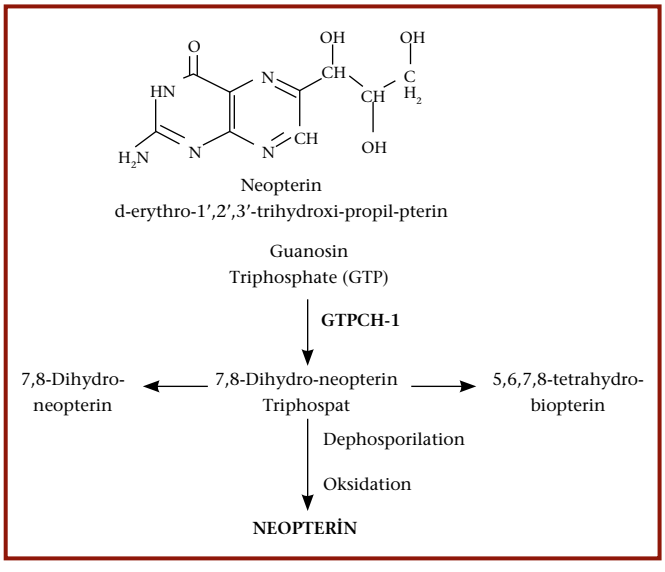

Figure 1. The molecular structure and synthesis of neopterin.

in chromatographic methods. Neopterin has been found in detecteable levels only in the body fluids of humans and primates. Measurable neopterin levels haven't been observed in healthy rats, guinea pigs and hamsters (15,16).

Neopterin is removed from the body in urine. When serum neopterin levels change, urine levels of neopterin change rapidly in parallel with serum (17). Therefore urine samples are often used for evaluation of the body neopterin levels. There is about 200-300 micromoles neopterin per mol creatinine in urine samples of healthy adults. An issue that must be considered in the evaluation of urinary neopterin levels is the presence of dihydroneopterin in urine. The measured neopterin value reflects the total pterins in some measurement methods, approximately 2-5 times higher results than real neopterin levels can be found because of the existence of dihydroneopterin (12). In order to neopterin levels in urine to be optimized according to the amount of urine, noepterin should be assessed in proportion to the level of creatinine. It is found that urinary neopterin/creatinine ratio in men were lower in than in women. It is stated that this difference occurs due to the calculation method because the urine creatinine concentration in men is higher than in women (18).

Laboratory measurements of neopterine

Measurement of neopterin concentrations in body fluids can be done easily under laboratory conditions due to its molecular structure is stable. However, it should be noted that neopterin is a light-sensitive molecule and losts it's fluorescence property when exposed to sunlight. For this reason, the samples must be protected from direct sunlight during transport and storage operations.

Main methods to used in the analysis of pterins are immunological measurement, electrophoretic methods and chromatographic methods. Of these methods, High Pressure Liquid Cchromatographed (HPLC) is the most preferred and more successful one than others in terms of analytical performance (19). Different from the HPLC method, thin layer chromatography (TLC), low-voltage or high-voltage single or two-dimensional electrophoresis, gas chromatography-mass spectrometry (GC-MS) and the mixture of colon/paper chromatography can be used. Nevertheless these methods are rarely used in clinical laboratories. These methods may be useful when considering the presence of an different pterin molecules and couldn't be determined by using normal HPLC methodology. Neopterin measurement in Gulhane Military Medical Academy Department of Biochemistry is done by HPLC method (20,21).

Except for chromatographic and electrophoretic techniques, the measurement of pterins can also be done by radioimmunoassay (RIA) method. Nevertheless it has a number of advantages, such as simultaneous measurement of multiple samples, small sample volume, no need for pretreatment of samples, being able to measure only one marker at one time and analytical performance's being dramatically low with regard to chromatographic methods are the main disadvantages of this method.

Neopterin in Organ Transplants

There are no routine and accepted practice criteria all over the world for the use of neopterin for clinical purposes yet. However, monitoring the levels of neopterin provides idea to the clinician about the patient's condition and course of the disease in some cases. Neopterin levels in body fluids of recipients with solid organ transplant such as kidney, liver, pancreas, heart, lung has clinical importance in predicting complications such as organ rejection and the development of secondary infection. A parallel relation between the measured neopterin levels and the severity of organ rejection has been found (22-23).

Serum neopterin levels of the patients who were transplanted kidney have shown a strong correlation with renal function. Serum neopterin concentrations are high in hemodialysis patients usually (22). Even though serum neopterin levels in the patients who have been followed in terms of renal function rise in the cases with increased cellular immunity, it also increases in renal function disorders such as acute tubular necrosis at the same time. Serum neopterin levels in kidney transplant patients should be evaluated along with serum creatinine concentration (6). Neopterin levels are generally low in renal transplant patients who haven't faced any problems in terms of tissue compatibility after transplantation. During episodes of graft rejection, neopterin levels are increased. Increased levels of neopterin have been reported before the emergence of the clinical signs of rejection of the graft in many patients (22-23).

Measurement of neopterin in patients liver transplanted has found useful in terms of showing the risk of immunological complications such as the development of tissue rejection and the development of secondary infection (25). But neopterin levels raised independently from immunological complications about the seventh days after liver transplantation. After this period it slowly decreases and if there isn't any complications, it remains at normal levels. It is reported that the rise in neopterin levels after heart transplantation has a sensitivity and specificity of 80-90% for organ rejection (26). It is thought that following up neopterin levels after heart transplantation may reduce the number of invasive myocardial biopsies. The increase of neopterin levels in pancreatic fluid after pancreas transplantation is observed before the emergence of the histological findings of tissue rejection (27-28).

Neopterin in Inflammatory Diseases

Urinary and serum neopterin levels were significantly increased in active periods of rheumatoid arthritis. This changes in neopterin levels actualize rapidly in parallel with the progression of the disease. Synovial fluid neopterin levels in RA (29) increases more specifically than the serum levels during inflammatory attacks. Neopterin levels have been increased in patients with active chronic inflammatory bowel disease (Ulcerative colitis, Crohn's disease). Neopterin levels of serum and urine are positively correlated with the severity of the disease (30). There is a similar relationship between the disease of Juvenile Crohn and neopterin (31). It is reported that there have been significantly high neopterin in the urine of the children with active celiac disease and there is a linear correlation between the anti-gliadin antibodies and neopterin. After starting gluten-free diet in these patients, neopterin levels are decreasing rapidly (32).

It is reported that neopterin levels increase in the early stages of type-I diabetes (33) and it remains at similar values with healthy controls in non-autoimmun diabetic patients. Similarly, in patients with autoimmune thyroiditis, serum neopterin has also been found elevated (34). In patients with active systemic lupus erythematosus, it is reported that neopterin levels are too high (35), this levels have decreased close to healthy controls in the patients whose clinical improvement provided with corticosteroid therapy. Persisting and increasing high levels of neopterin values has been observed in patients with poor prognosis.

Neopterin in Malignant Diseases

Neopterin levels in hematologic malignancies vary depending on the stage of the disease. Neopterin levels can also be elevated in patients at remission stage. The most highest neopterin levels have been observed in the patients with non-Hodgkin's lymphoma and chronic lymphoblastoid leukemia. If the neopterin levels progress at high levels in the patients with Hodgkin's disease, surveillance is expected to be poor (36-38).

In patients with ovarian cancer (39), the release of neopterin increases by the progression of tumor stage. There is a similar correlation with cervical and uterine malignancies as well, although weaker than the ovarian cancer (40). Neopterin levels also provide useful information for the differential diagnosis of uterine sarcoma, and benign uterine myoma (41). In patients with cervical, ovarian and prostate cancer patients, a statistically significant relationship between neopterin concentrations and prognosis has been reported (39-40).

Neopterin levels are found high in 75% of patients with active lung cancer. There is no significant difference in terms of neopterin levels among histological types of lung cancer (42).

Neopterin levels have been found high approximately 40-50% of patients with gastric and colorectal cancer, and no relation has found between neopterin levels and the stage of the disease (43). Among GIS malignancies patients with pancreatic carcinoma, neopterin levels were higher than in other malignancies. A high increase in concentration of neopterin has been observed in carcinomas of the biliary system. However, there is a weak correlation between high neopterin levels and the stage of the disease in these malignancies (43). In hepatocellular carcinoma, a positive relationship has been found between neopterin levels and the size of the tumor (44).

High levels of neopterin has been found in a small proportion of patients with breast cancer (about 20%). There is a weak correlation between neopterin levels and stages of the disease (45). Neopterin levels are elevated in the presence of metastases in these patients. The concentration of neopterin shows

a lower increase in larynx, oral cavity, pharynx, paranasal sinuses, and head and neck cancers just as in breast cancers (46). Monitoring levels of neopterin provides useful information in determining visceral metastases in patients with malignant melanoma (47). Neopterin is at normal levels in the patients who have excised malignant melanoma.

In all of these malignancies, the increase in neopterin levels is a significant marker for deterioration of the tumor, metastasis or death risk of the patients (43-47). Monitoring of the levels of neopterin is found very useful from the point of the decisions to be given on second-look laparotomy especially in patients with ovarian carcinoma of malignant diseases (39). High levels of neopterin is recognized as an important signal for residual tumors after the second-look operation.

Neopterin in Various Clinical Conditions

Almost all clinicopathological cases, in which the cellular immune response has an active role, rise of neopterin levels can be reported. The cellular immune system plays an active role in all kinds of activities such as cell death and removal of cell debris from environment arising after cellular damage, removal of the molecular structure from the body that is foreign to the organism. In this regard, in almost all infectious diseases, according to the severity of the clinical conditions, neopterin levels are expected to rise. Thus, in the infectious diseases with high mortality rates, monitoring the levels of neopterin is thought to be useful in terms of estimating the clinical course of the disease. In the study on a group of Crimean-Congo hemorrhagic fever, it is reported that neopterin levels give more useful information than the tests such as bleeding time, platelet count, serum liver enzyme levels, to predict the risk of mortality (48). In a study conducted in a group with brucellosis disease, in order to determine whether the treatment is successful, in terms of increasing morbidity in chronic infectious diseases, as another problem clinicians faced by, neopterin levels are reported to give more useful information than the routine tests in monitoring the success of therapy (49). Neopterin, can also be used as a helping marker in the diagnosis of infectious diseases that are difficult to diagnose. Regarding this issue, in a study conducted in 2005, on patients with tuberculous pleurisy, the measurement of neopterin levels of urine samples and pleural fluid was reported to be a useful marker in the differential diagnosis of pleurisy (50). Other study in patients with tuberculous pleurisy, urine serum neopterin levels was found to be significantly higher than tuberculosis and non-tuberculosis healthy controls (51).

In cytotoxic tables that acute and chronic cell injury and cell death takes place in physiopathology, since cellular immunity takes active roles, an increase in the levels of neopterin is expected in such statements. Urinary neopterin levels increased in non-alcoholic steatohepatitis and chronic viral hepatitis, but it is reported that this increase dosesn't correlate with the histopathologic stage of the disease (52). In this type of pathologies that cell damage occurs in chronic process, since the cytotoxic effect has been extended over a period of time and the increase in the number of damaged cells is paralel with histopathological findings, no relation between neopterin levels and the stage of the disease can be found. However, neopterin levels increase in accordance with the histological grade in experimental studies on acute cytotoxic pathologies such as acetaminophen-induced hepatotoxicity and nephrotoxicity (53-54). Besides the use of clinical purposes, neopterin provides useful information to researchers in the cases where cellular immunity suspected to have role in etiopathology. In a study conducted on preeclampsia whose etiopathogenesis has not fully understood yet, neopterin levels in serum samples of pregnant women with preeclampsia and cord blood samples taken after the birth are significantly higher than healthy controls (55). These findings suggest the role of cellular immune response in the pathogenesis of preeclampsia.

First reaction of the organism's to any disease or pathogens is inflammatory response. Inflammatory response is involved in almost every clinicopathologic process. For this reason, a number of indicators are severely needed to determine the degree of inflammatory response by researchers and clinicians. Nowadays, cytokine levels in body fluids are often used for the evaluation of the responses of the immune system to stimuli. But because of the reasons such as the vast majority of cytokines are biologically labile, quickly cleaned from the circulation after releasing and showing local effects in most cases, researches to find more stable and more useful indicators to monitoring inflammatory reactions are continuing.

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