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ORIGINAL ARTICLE / ÖZGÜN MAKALE

NEOPTERIN: A POSSIBLE BIOMARKER IN GASTROINTESTINAL CANCER

NEOPTERİN: GASTROİNTESTİNAL KANSERDE OLASI BİR BİYOBELİRTEÇ

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ABSTRACT

Objective: *Cancer, one of the major causes of death in the world, is described as a hyperproliferative disorder. Inflammation which is a primary innate immune response to perturbed tissue homeostasis, has become an important hallmark of cancer. Neopterin is produced by activated macrophages upon stimulation with proinflammatory cytokines. It is immune activation biomarker increased in different disorders associated with immune activation, including cancer. We aimed to determine the neopterin levels in gastrointestinal cancers (GIC) and to evaluate the differences among the subgroups.*

Material and Method: *The study included 108 patients with GIC and* 25 *healthy controls.* Patients were divided into three subgroups. The first group consisted of 40 individuals with gastric cancer, the second group consisted of 40 individuals with colorectal cancer, and the third group consisted of 28 individuals with pancreatic, liver or esophageal cancer. Serum *neopterin levels were measured by ELISA.*

Result and Discussion: *Neopterin was significantly higher in all patient groups compared to controls ($p<0.05$). It was found that* HDL-cholesterol, albumin and total protein levels were significantly lower in all patient groups compared to controls. *Our results show that neopterin levels are elevated in gastrointestinal cancers. Therefore, neopterin may be a potential predictive marker in GIC patients*.

Keywords: *biomarker; colorectal cancer; gastric cancer; inflammation; neopterin*

ÖZ

Amaç: *Dünyadaki başlıca ölüm nedenlerinden biri olan kanser, hiperproliferatif bir bozukluk olarak tanımlanmaktadır. Bozulmuş doku homeostazına karşı doğal bir immün yanıt olan inflamasyon, kanserin önemli bir özelliği haline gelmiştir. Neopterin, proinflamatuvar sitokinlerin uyarımı ile aktive olan makrofajlar tarafından üretilmektedir. Neopterin, kanser dahil immün aktivasyon ile ilişkili farklı bozukluklarda artan*

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immün aktivasyon biyobelirtecidir. Çalışmamızda gastrointestinal kanserlerde neopterin düzeylerini tayin etmeyi ve alt gruplar arasındaki farklılıkları değerlendirmeyi amaçladık.

Gereç ve Yöntem: *Bu çalışmaya 108 gastrointestinal kanserli hasta ve 25 sağlıklı kontrol dahil edildi. Hastalar üç alt gruba ayrıldı. Birinci grup mide kanserli 40 kişi, ikinci grup kolorektal kanserli 40 kişi ve üçüncü grup pankreas, karaciğer ya da özofagus kanserli 28 kişiden oluştu. Serum neopterin düzeyleri ELISA ile ölçüldü.*

Sonuç ve Tartışma: *Neopterin, kontrollerle karşılaştırıldığında tüm hasta gruplarında anlamlı olarak daha yüksekti ($p<0.05$). Tüm hasta gruplarında HDL-kolesterol, albümin ve total protein düzeyleri kontrol grubuna göre anlamlı derecede düşük bulundu. Sonuçlarımız neopterin düzeylerinin, gastrointestinal kanserlerde arttığını göstermektedir. Dolayısıyla, neopterin, gastrointestinal kanserli hastalarda potansiyel tahmini bir belirteç olabilir.*

Anahtar Kelimeler: *biyobelirteç; inflamasyon; kolorektal kanser; mide kanseri; neopterin*

INTRODUCTION

Cancer is defined as a hyperproliferative disorder that includes morphological cellular transformation, uncontrolled cellular proliferation, dysregulation of apoptosis, angiogenesis, invasion, and metastasis [1]. Currently, cancer is one of the main causes of death, 7.6 million cancer deaths occurred in 2008 and it is predicted that cancer related mortality could reach 13.1 million deaths by 2030 [2]. Gastrointestinal cancers which are a group of highly aggressive malignancies with heavy cancer-related mortalities, are one of the most common cancer types in the worldwide [3,4]. Esophagus, stomach and colorectal cancers are the most important digestive cancers because of their low survival rates and high mortality rates. It was reported that 5-year survival rates for stomach cancer are 11.5 and 16.4% for males and females, and for colorectal cancer are 45 and 39% in females and males respectively. Therefore these cancers have attracted special attention [4]. Interaction between cancer cells and the immune system is becoming an increasingly important issue. Especially, the newly developed active immunotherapies make inflammation and immune activation markers highly interesting [5]. Inflammation is a primary innate immune response to perturbed tissue homeostasis [6]. Neopterin is an established biomarker of systemic adaptive immune activation [7]. It is a pteridine molecule [8] which 253 dalton molecular weight [9]. It is produced by activated macrophages upon stimulation with proinflammatory cytokines, particularly interferon-γ (IFN-γ) [10-13]. It is synthesized from guanosine triphosphate (GTP) in a reaction catalyzed by the enzyme GTP cyclohydrolase I (GCH-I) [5,9,14-16]. GTP first transforms into an intermediate product called 7,8-dihydroneopterin triphosphate by GCH-I. This is the rate-limiting step of the pathway leading to the formation of 5,6,7,8-tetrahydrobiopterin. GTP cyclohydrolase I can be induced by IFN-γ in various cells and species. The subsequent enzymes of the pathway such as 6-pyruvoyl-tetrahydropterin synthase, which also is relatively insensitive to IFN-γ are not expressed by human monocyte-derived macrophages and dendritic cells unlike most other cells. As a result, neopterin generates via dephosphorylation of 7,8-dihydroneopterin triphosphate and oxidation [9,14,16].

Neopterin can exist for a long time in the body fluids such as blood, urine, and cerebrospinal fluid. Its level is stable without inactivation and degradation. It is also an appropriate marker that can reflect the cellular immune status induced by the lymphocyte macrophage system [17]. Due to its early and highly change to various pathological situations, neopterin in human body fluids is considered a reliable marker as monitoring processes, development and prognosis of many diseases including immune disorders, inflammations, and coronary artery diseases [18]. The aim of the present study was to examine the changes in neopterin levels in the serum as a possible biochemical marker in patients with gastrointestinal cancer and the differences among the subgroups.

MATERIAL AND METHOD

The study included 108 patients (38 women, 70 men) with gastrointestinal cancer from Ankara Oncology Educational and Research Hospital and 25 healthy controls with no clinical symptoms of any disease (15 women, 10 men). The study was approved by the Ankara Oncology Educational and Research Hospital Ethics Committee to be in accordance with the Helsinki Declaration. All of the patients gave written informed consent. The mean age and quetelet index (QI) in patients were 54.17±1.376 years, 24.54±0,31 and in controls was 43±2.68 years, 25.58±0.98. Patients were divided into three subgroups. The first group consisted of 40 individuals with gastric cancer (9 women, 31 men), the second group consisted of 40 individuals with colorectal cancer (13 women, 27 men), and the third group consisted of 28 individuals with pancreatic, liver or esophageal cancers (16 women, 12 men) (Table 1).

Blood samples of patients were collected before the operation and than serum was seperated immediately. All blood samples were centrifuged for 10 min at 1000 g at +4ºC and supernatants were stored at –80°C till assay. Serum neopterin concentrations were determined according to the manufacturer's instructions by a commercially available Elisa (DRG Diagnostic) kit. Serum lipids were measured spectrophotometrically on the Roche-Hytachi P-800 autoanalyzer by using a commercial kit (Roche, Mannheim, Germany).

Data was expressed as mean ± standard error (SE). The SPSS statistical package programme (SPSS, version 16.00) was used for statistical analyses. Student's t-test was used to compare results between the all patients and control groups. Multiple regression and Pearson correlation analyses were carried out to assess to association of pairs of measured parameters. Statistical significance was set at $p<0.05$.

RESULT AND DISCUSSION

Cancer is the second leading cause of death in the world and one of the main fields of medical research. Although there is now a greater understanding of biological mechanisms of uncontrolled cell growth, invasiveness and metastasization, the multistep process of cancer development and evolution is still incompletely understood [2]. Cancer is a multifaceted condition, in which a senescent cell begins dividing in an irregular manner due to various factors such as DNA damage, growth factors and inflammation [19]. Inflammation has become an important hallmark of cancer, and enhanced inflammatory mediators are associated with poor prognosis in patients with cancer [20]. Inflammatory responses play decisive roles at different stages of tumor development, including initiation, promotion, malignant conversion, invasion, and metastasis [21]. Interferon-γ is a proinflammatory cytokine with pleiotropic functions [22]. Neopterin is synthesized mainly by monocytes/macrophages after induction by IFN-γ and is considered to be a marker for activation of cellular immune system [23]. Neopterin is a biomarker of immune activation increased in different disorders associated with immune activation, including cancer [24]. Increased levels of neopterin in the serum and urine have been described in several malignancies such as lung [25], endometrial [26], thyroid [8] and breast cancers [27]. It was shown that neopterin levels in patients with malignant breast disease were significantly higher than in the benign group [28]. One of the previous study demonstrated that neopterin in patients was increased only in a minority of patients with breast carcinoma [29]. Thein et al. reported that neopterin was significantly elevated in prostate cancer and exhibited discriminatory capacity. In contrast, neopterin had no discriminatory power to detect breast cancer [30].

Gastrointestinal cancers with high cancer-related mortalities, is a worldwide problem [3,4]. Inflammation and inflammatory conditions have been associated with pancreatic cancer risk [31]. Talar-Wojnarowska et al. observed that neopterin levels enhanced in patients with pancreatic cancer [32]. Although some studies reported that neopterin was associated with increased risk of several cancers and all-cause mortality, Huang et al. found that neopterin was not significantly associated with pancreatic cancer risk [33]. Primary and secondary liver tumors are associated with poor prognosis. It was reported that urinary neopterin is increased in patients with liver tumors [34]. Chronic inflammation and immunity are suggested to play a role in the pathogenesis of colorectal cancer [35]. Previous investigations demonstrated that urinary neopterin was significantly increased in colorectal carcinoma patients [36,37]. It was observed that highly elevated, but not moderately elevated, total neopterin concentrations were associated with higher risk of colorectal cancer [35]. Although gastric cancer incidence has been declining over the last few decades, they remain the second leading cause of cancer-related deaths worldwide [19]. It was found that neopterin levels enhanced in patients with gastric cancer [38]. Engin et al. reported that the overall increase in postoperative neopterin levels of gastric cancer

cases was highly significant, although the extent of tumor invasion might have gradually decreased the macrophage response to surgical trauma [39].

Table 1. Demographic and biochemical parameters in all groups

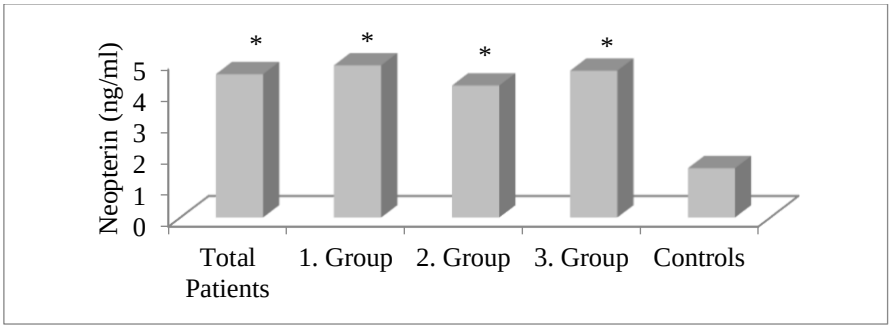
	Total Patients (n=108)	1. Group (n=40)	2. Group (n=40)	3. Group (n=28)	Controls (n=25)
Age, years	54.17±1.376	52.62±1.98	55.25±2.49	54.82±2.76	43±2.68
Gender, female/male	38/70#	9/31#	13/27#	16/12#	15/10
Quetelet **index, kg/m^2**	24.54±0.31	24.39±0.53	24.73±0.52	24.51±0.58	25.58±0.98
HDL-C (mg/dl)	31.55±1.08*	31.08±1.86*	32.27±1.85*	31.21±1.90*	44.08±1.94
Uric acid (mg/dl)	4.52±0.39	4.08±0.28	4.21±0.33	5.58±1.36	4.24±0.21
Albumin (g/dl)	3.35±0.09*	3.21±0.14*	3.63±0.18*	3.14±0.14*	4.41±0.10
Total Protein (g/dl)	6.09±0.11*	5.99±0.16*	6.26±0.20*	5.98±0.22*	7.49±0.10
TNF-α (pg/ml)	14.90±1.25*	16.28±2.23*	11.46±1.70*	17.86±2.59#	7.53±0.70
Neopterin (ng/ml)	4.56±0.39*	4.84±0.74*	4.20±0.68*	4.67±0.45*	1.57±0.13

*significant difference from controls (p<0.001)

significant difference from controls (p<0.05)

In this study, we evaluated the levels of neopterin in gastrointestinal cancer patients and controls. Our study demonstrated that neopterin levels were significantly higher in total patients and subgroups compared to controls (p<0.001). It was also found that there was no statistically significant difference between the subgroups (Figure 1). Elevated levels of neopterin in patients with GIC may be accounted for by long-term stimulation of the immune system. Our results are in agreement with other studies which reported that neopterin levels enhanced in cancer patients. There was a significant difference between patient and control groups in terms of gender. However, it was observed that gender had no effect on neopterin levels. In our study, neopterin levels in patients whose quetelet index is smaller than twenty-five were increased compared to those of QI is equal and bigger than twenty-five in terms of QI (p=0.001). Enhanced neopterin levels in cancer can amplify the proinflammatory response which is involved in the pathogenesis of cancer-associated weight loss, but a specific role of neopterin in this condition is still unclear. TNF-α was found to induce neopterin generation despite the fact that any direct effect of the cytokine on neopterin generation is almost absent [14]. In our previous study, we measured the levels of proinflammatory cytokine TNF-α in these GIC patients [40]. When we

reassessed TNF-α levels for this study, it was found that TNF-α levels were significantly higher in total patients and subgroups compared to controls (respectively, p=0.000, p=0.000, p=0.038, p=0.001), (Table 1). There was no correlation between neopterin and TNF-α levels in total patients and subgroups, and it was found a positive correlation in controls (p=0.004).



*significant difference from controls (p<0.001)

Figure 1. Neopterin levels in total patients and subgroups

Inflammation and malnutrition can suppress the synthesis of albumin [41,42] and affect the levels of serum lipids. [43]. Uric acid promotes inflammation, cell proliferation, and migration by inducing COX-2 expression. However, the role of uric acid in cancer is still debated [44]. Recent evidence has shown that elevated serum uric acid is associated with excess cancer risk, recurrence, and mortality. Hyperuricemia is associated with increased risk of colorectal, breast, prostate, and other cancers [45]. It was found that albumin, total protein and HDL-cholesterol levels of the total patients and subgroups were significantly lower than the controls (p<0.001), but no significant difference was found in lipid values and uric acid levels (Table 1). In the present study, uric acid levels seem to be increased, however it was not statistically significant. There was a negative correlation between neopterin and albumin levels (p=0.002) ,and also a positive correlation between neopterin and VLDL levels (p=0.003) in total patients. In the gastric and colorectal cancer patients' groups, there were positive correlations between neopterin and VLDL levels (p=0.045, p=0.005, respectively) and also uric acid levels (p=0.020, p=0.001, respectively). Our findings suggest that serum albumin, total protein and HDL cholesterol levels seem to be altered in GIC, and neopterin may contribute to this process.

Consequently, the detection of potential biomarkers for cancer can be useful for diagnosis, prognosis, and therapeutics. The newly developed active immunotherapies make inflammation and immune activation markers highly interesting. Neopterin is considered as a noteworthy marker that reflect the immune activation status. Elevated neopterin concentrations are observed in different types of cancer. Therefore, neopterin that is an established biomarker of immune activation may be a potential predictive marker in GIC patients.

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