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Association of Urinary Neopterin and Total Neopterin with Stages II and III Breast Cancer

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ABSTRACT

Breast cancer is the most prevalent form of cancer in women worldwide and Iraq every year. Many clinical, diagnostic and pathological techniques have been in front to get early detection of breast tumors**.** The objective of this study was to assess urine neopterin and total neopterin levels in patients with stages II and III breast cancer**.** This study is a cross sectional study that was conducted between July 2019 to October 2019 in Medical City Hospital in Baghdad, Iraq. A total of 50 patients newly diagnosed with breast cancer were divided into two groups according to the obtained results in which; 30 patients newly diagnosed and untreated with stage II breast cancer, and 20 patients newly diagnosed and untreated with stage III breast cancer. Levels of neopterin and total neopterin were measured in all participants by High Performance Liquid Chromatography (HPLC) technique. The markers (neopterin, 7, 8-dihydroxy neopterin and total neopterin) in urine of breast cancer patients showed no significant correlations between the two stages breast cancer patients. A no significant correlation was found for neopterin between stages II and III in the urine of breast cancer patients in this study (P- value=0.482and, r=0.30).Total neopterin on the other hand showed no significant correlation between stages II and III in urine (P- value=0.962, and r= 0.2). The current study concluded the possibility of the potential clinical use of neopterin and total neopterin as useful markers in urine of breast cancer patients for diagnosing the different stages of the disease.

Keywords: Neopterin, Total neopterin, Breast cancer.

Introduction

Breast cancer is a group of diseases that cause cells in the body to change and spread out of control. Most types of cancer cells eventually form a lump or mass called a tumor, and are named after the part of the body where the tumor originates. Most breast cancers begin either in the breast tissue made up of glands for milk production, called lobules, or in the ducts that connect the lobules to the nipple. The remainder of the breast is made up of fatty, connective, and lymphatic tissues [4].

Neopterin:the aromatic pteridine; neopterin is produced by monocytes and macrophages in response to the stimulatory effect of interferon-gamma (IFN-γ) that is secreted by activated T-

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cells. Its production may provide information about the status of cellular immunity, so neopterin acts as a marker for immune system activation. Neopterin is a highly fluorescent pterin compound [1]. Increased concentrations of urinary and serum neopterin have been reported in a variety of cancers including ovarian, cervical, endometrial and vulvar carcinomas, and uterine sarcomas. A significant positive correlation was obtained between high neopterin levels with advanced tumor stage. Also, high neopterin concentrations in various body fluids were observed in patients with viral infection or an autoimmune disease and in patients experiencing allograft rejection [8].

Total neopterin (T.N), is a by-product in the biopterin biosynthesis and an indicator of activation of the cellular immune system [3]. The measurement of neopterin and total neopterin (which is the combined value of 7, 8-dihydroneopterin and neopterin), are of almost equal potential importance for clinical diagnosis and have gained great interest in many studies regarding cancer patients recently. However, when measuring total neopterin, which includes oxidation of 7, 8-dihydroneopterin to neopterin, more strict requirements of sample collection and handling are necessary to avoid degradation of the 7, 8- dihydro derivative [5]. This relationship has been shown recently in studies of exercise and impact-induced injury during intense contact sports. Therefore, neopterin and total neopterin, could provide a more comprehensive analysis of clinical inflammation than neopterin alone [2]. Evidence suggests that measuring either neopterin or 7, 8 dihydroneopterin alone cannot provide a complete picture of immune activation [2].

Patients and Methods

Methods

Study Participants

A cross sectional study was conducted between July 2019 to October 2019 in Medical City Hospital in Baghdad, Iraq. Informed consent forms were obtained from all participants before having them take part in the study. The study protocol was accepted by the Ethics Committee of the College of Medicine/Al-Nahrain University. A total of 50 women diagnosed with breast cancer were divided into two groups according to the obtained results in which; 30 patients newly diagnosed and untreated with stage II breast cancer. And 20 patients newly diagnosed and untreated with stage III breast cancer.

Laboratory Assays

Five milliliters of urine samples were collected and they were covered with aluminum foil and stored at (-20 ◦C) and dark condition until use for further evaluation.

Measurement of Urinary Neopterin and Total Neopterin

Samples urine were prepared and kept in darkness, to prevent possible oxidative loss of 7, 8-dihydroneopterin and other pterins derivative from UV light.

Urine samples were thawed and diluted to 10 in 40 with phosphate buffer (20mM (NH4)3PO4, pH2.5) for neopterin and creatinine determination.100 µL was transferred to an autosampler vial and the sample were passed through 2.5 um disposable filter. Then 20 µL were injected onto HPLC.After neopterin peak separated on the first phenomenex, C-18, 3microm particle size (50x 4.6mm, id) column. 4-Using deionized water as a mobile phase, the free neopterin was eluted

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within 2minutes.Then the HPLC system was switched onto a second similar phenomenex column C-18.2 minutes after of the first injection to eluted other neopterin produced by oxidation step resulted from convert 7, 8 -dihydroneopterin and other pterins to the fluorescent neopterin.The other oxidized neopterin were eluted with sodium dodecyl sulphate 0.1 g/L in deionized water (adjusted to pH 4.0 with trifluoroacetic acid, TFAC) at flow rate of 1.0 ml/min the second neopterin were eluted at 4.3minute.The concentration of neopterin was quantitatively determined by comparison of the peak area of the standard with that of the samples under the same separation condition

Calculation:

Conc of sample ug/ml=Area of sample/Area of standard xconc. Of standard x dilution Factor.

7, 8-dihydroneopterin was obtained from the following equation:

Total Neopterin = Neopterin +7, 8-dihydroneopterin

Statistical Analysis

The data of the study were analyzed using the SPSS software 20 and Microsoft excel program (2010). Numeric variables were expressed as mean+ SD and all statistical comparisons were made by means of independent t-test and ANOVA test with $P<0.05$ was considered statistically significant..

Results:

The concentrations of neopterin, 7,8-dihydroxy neopterin (7,8.D.H.NP) and total neopterin in urine of stage II and stage III breast cancer patients (30 patients Stage II and 20 patients StageIII) are given as mean values with standard deviations in Table 1, and Figures 2 and 3. Mean age of breast cancer women with stage II was 44 years, and breast cancer women stage III women 44 years of age.

Table 1: Demographic characteristics of the study population

Variables	StageII	StageIII
Age (years) 30-50	Min 30 Max 50	Min 36 Max 50
Mean	44 years	44 years
Neopterin, nmol/L	139.23± 26.95 µmol/mol creatinine	138.55±34.41 µmol/mol creatinine
Total Neopterin, nmol/L	336.50± 54.56 µmol/mol creatinine	316.00±72.21 µmol/mol creatinine
7,8Dihydroneopterin, nmol/L	197.27± 33.60 µmol/mol creatinine	177.74±40.73 µmol/mol creatinine

SD: standard deviation,

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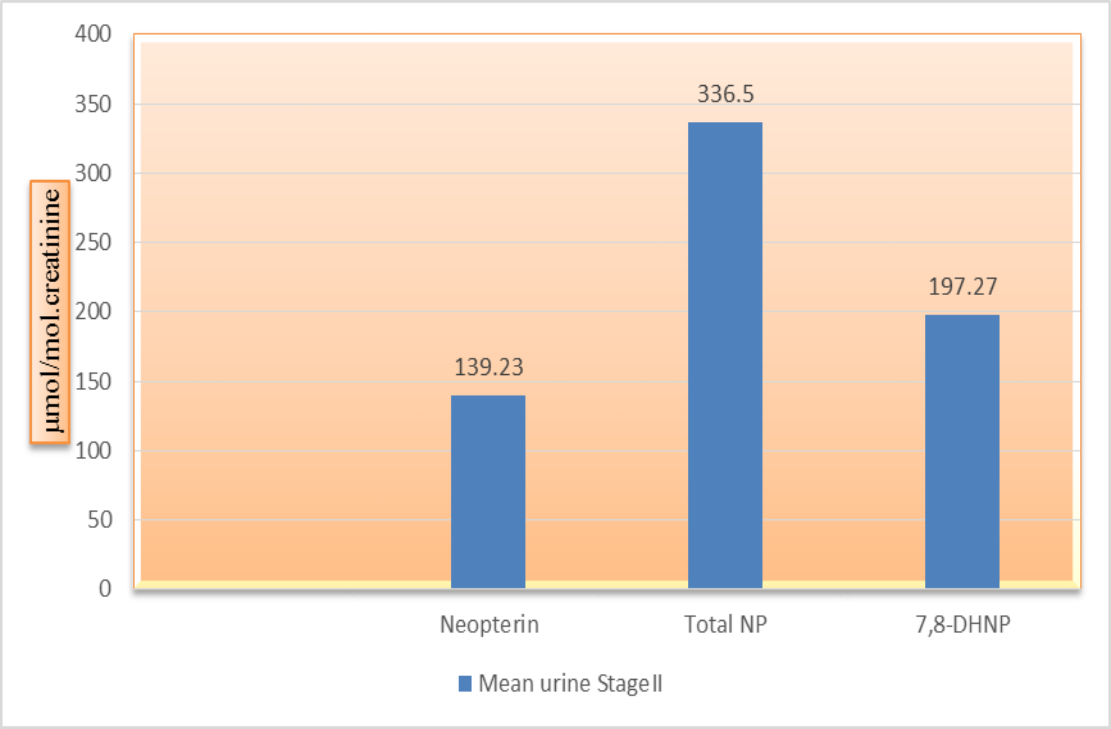

Figure 1: Histogram showing the mean study markers in urine for stage II breast cancer patients

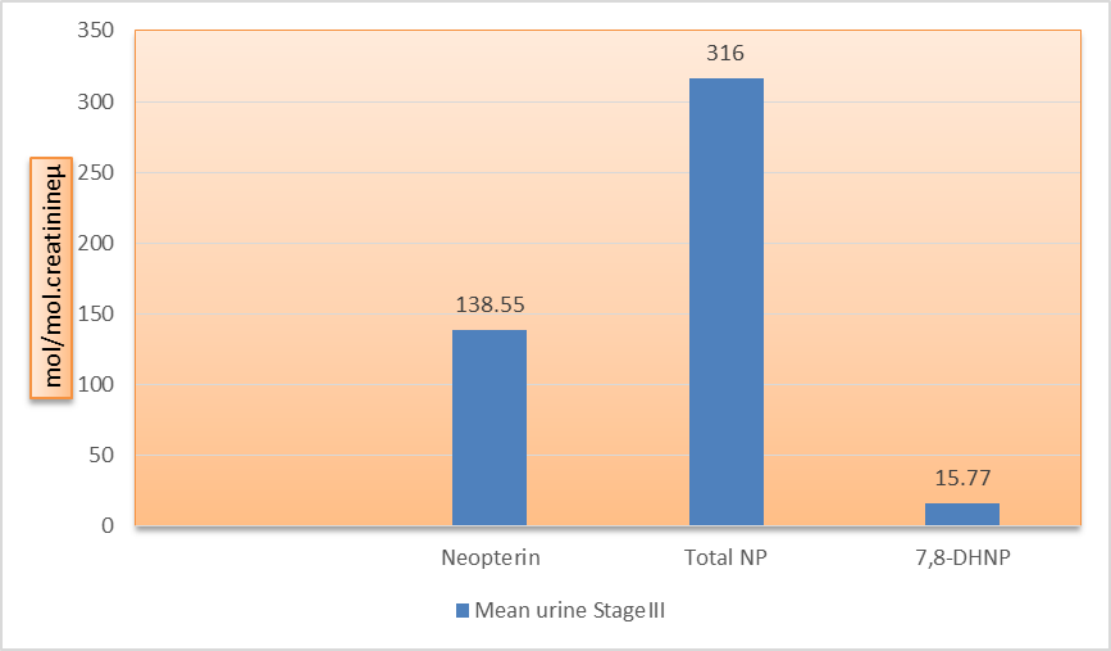

Figure 2: Histogram showing the mean study markers in urine for stage III breast cancer patients

Discussion:

In this study the level of urine neopterin in stage II breast cancer patients was relatively in the upper higher limits (mean + SD =139.23± 26.95µmol/mol creatinine). While, the urine neopterin level was no significantly higher in stage III (mean + SD=13.63+ 5.59 nmol/L) than stage II. Due

to this significant difference, an indication of the severity of the dis138.55±34.41µmol/mol creatinine)ease may be obtained by measuring urine neopterin levels in breast cancer patients.
The results in this study are in agreement with previous studies in which the neopterin concentration was found to be dependent on the stage of malignancy. Also, that there is a relationship between neopterin concentration in the urine and the approximate total tumor mass [7].
Another study [2] showed that neopterin was found to be significantly elevated in patients with advanced stages and grade III tumors.
Oxidative stress in humans may be indicated by an increase in neopterin concentrations [9]. It was found that neopterin can enhance the oxidative potential of the reactive oxygen species (ROS) that are produced from immune competent cells [9]. Since, (ROS) have been implicated in initiation and the promotion of carcinogenesis [10]. Therefore, the value of higher neopterin concentrations could predict disease progression and death in malignant diseases and it could be related to the capacity of neopterin derivatives to induce ROS and oncogene expression [9].
The levels of total neopterin obtained from this study were no significantly in stage III breast cancer patients than the level in urine of stage II breast cancer patients given by the values (mean + SD =316.00±72.21µmol/mol creatinine and 336.50± 54.56µmol/mol creatinine respectively)
So, the levels of 7, 8-dihydroneopterin obtained in this study were also no significantly higher in urine of stage III breast cancer patients than that of stage II breast cancer patients (mean + SD= 197.27± 33.60µmol/mol creatinine and 177.74±40.73µmol/mol creatinine) respectively.
This elevated value of total neopterin especially in stage III breast cancer patients may be due to the increased inflammation and oxidative stress in the more advanced stage of breast cancer. Since, the antioxidant activity of 7,8-dihydroneopterin has led to the proposal that it is generated during macrophage activation with the purpose of self-protection within the highly oxidizing environment of an inflammatory site [1].
A review of different studies on neopterin and total neopterin provided by [6] suggested that measuring either neopterin or 7,8-dihydroneopterin alone can't provide the complete picture of oxidative stress or immune activation in an individual. Therefore, the combination of both biomarkers gives a more robust mechanism of the inflammatory process, with an analysis of neopterin being a measure of the oxidative status within the cells, and 7,8- dihydroneopterin being a measure of interferon-mediated cellular activation.
To our knowledge measurements of 7,8-dihydroneopterin and hence, total neopterin in breast cancer patients has not been investigated to date. This may be due to the relative ease of measuring neopterin alone. Therefore this study has shown the importance of measuring total neopterin rather than neopterin alone, this is due to the no significant levels and differences between 7,8-dihydroneopterin between stage II and stage III breast cancer patients.

Conclusions:
The data obtained in this study shows the possibility for the potential clinical use of neopterin and total neopterin as therefore, using a urine sample as a non-invasive and easy to obtain sample could be used solely to measure these markers. diagnosing the different stages of the disease.

Conflict of interest:
The authors declare that there was no conflict of interest regarding the publication of this paper.

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