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Arginase activity and nitric oxide levels may be considered as tumor markers in breast cancer

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ABSTRACT

To investigate the relationship of breast cancer with serum arginase activity and nitric oxide levels. Arginase (L-arginine ureohidrolase, EC. 3.5.3.1) is the last enzyme of urea cycle which converts arginine into urea and ornithine. nitric oxide, a product of L-arginine and nitric oxide synthetase reaction, is a hormone, a reactive oxygen species, neurotransmitter, mediator, cytoprotective molecule, and the only endogenous molecule that acts as a cytotoxic molecule. This study was done at Gaziantep University Research Hospital with 30 breast cancer patients (30-77) and with 34 healthy people (30-75) to diagnose breast cancer. The majority (n=28) was ductal while the rest were medullary and papillary cancers. Serum arginase activity was measured by thiosemicarbazide diasetilmonoksim urea method in U/L that was modified. Also, serum nitric oxide levels were measured by the Griess method in terms of mmol/L. Serum arginase activity was determined as 17.8±2.5 (X±SE) U/L in the patient group and 6.8±0.9 U/L in the control group; the cancer patients showed a significant increase (t=3,649, p <0.01). Serum nitric oxide levels were found 139.4 ± 7.4 mmol / L in the breast cancer group, and 95.9 ± 7.6.1 mmol/L in the healthy group. nitric oxide levels were found to be significant in the analysis (t=4,197, p<0.001). In this study, a significant increase in serum arginase and nitric oxide activity was observed for the breast cancer patients, and it was concluded that both could be important for the diagnosis of breast cancer and for its treatment.

INTRODUCTION

Nitric oxide (NO) is one of the simplest biologically active molecules in biochemistry that is synthesized from L arginine amino acid by the enzyme nitric oxide synthase (NOS). It is the only endogenous molecule that acts as a mediator, a hormone, a reactive oxygen species (ROS), neurotransmitter, cytoprotective and cytotoxic molecule (Akyol *et al*., 2004). A decrease in NO production stimulates oxidative phosphorylation and increases peripheral oxygen uptake (Szabo C *et al*., 1995). It is caused by lipid peroxidation, nitrosylation of molecules, inactivation of sodium channels, and redox reaction with metals such as iron and copper (Mayer and Hemmens, 1997).

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The enzyme arginase (EC.3.5.3.1) hydrolyses L-arginine amino acid to urea and ornithine using two isoenzymes (Levillain *et al*., 2005; Vockley *et al*., 1996). While ariginase I among isoenzymes mostly found in liver and functions in the synthesis of urea, arginase II plays a mitochondrial enzyme in the extra-hepatic tissues (Srivastava and Ratha, 2010; Wu and Morris, 1998). Arginase II isozyme which synthesizes glutamic acid, proline and ornithine metabolite which the substrate in the synthesizes of polyamines (Russell and McVicker, 1972; Tabor and Tabor, 1984). The relationship between the NOS and arginase involves not only the use of the same substrate (Hecker *et al*., 1995; Meyer *et al*., 1997). In healthy individuals, sufficient arginase activity can limit the use of arginine for the synthesis of NO [Currie *et al*., 1979; Vodovotz *et al*., 1994). In many studies, macrophage-

induced arginase activity in wounds has shown to be involved in tissue healing and protecting against inflammation and infection. In addition, the enzyme arginase removes arginine from the media by using it and thereby affects the level of NO (Meyer *et al*., 1997; Shearer *et al*., 1997).

MATERIAL AND METHODS

Participants: The present study included 31 patients aged from 30 to 77 (48.03 ± 2.51) years and diagnosed with breast cancer in Gaziantep University Oncology Hospital and 34 healthy volunteers aged from 30 to 75 (48.66 ± 2.55) years. The difference between the mean ages was not significant (p> 0.05). The majority of breast cancers were ductal carcinoma (n = 28) and the remainder were medullary and papillary carcinomas. Sera obtained from blood samples taken on while fasting in the morning were stored at -80^{o} C until the day of operation. The study was conducted after obtaining approval from local ethics committee of Gaziantep University on 30.06.2008.

Test methods: Arginase activity in the serum was studied spectrophotometrically with a modified method of Thiosemicarbazide Diacetyl Monoxiıne Urea (TDMU) (Ahi *et al*.,1999; Geyer and Dabich, 1971). NO levels were also measured spectrophotometrically by the Griess method. With this method, nitrous and nitrate levels measured and NO level calculated indirectly (Cortas and Wakid, 1990).

Statistical methods: The data were evaluated using SPSS software. Student "t" test and Mann-Whitney test were used for dependent and independent groups, respectively. In addition, Pearson correlation analysis was performed (Habbema *et al*., 2002; Lang and Secic, 1997).

RESULT AND DISCUSSION

There was no significant difference between the mean ages of 31 breast cancer patients aged from 30 to 77 (48.03 ± 2.51) years and 34 healthy volunteers aged from 30 to 75 (48.66 ± 2.55) years (p> 0.05). The mean arginase activity [arithmetic mean + standard error (x ± SE)] in the healthy group was 6.88 ± 0.9 (0.4-26) U/L, whereas this ratio was 17.88 ± 2:57 U/L in the breast cancer group. The comparison of the arginase activity between breast cancer patients and healthy control group showed three-fold increase in arginase activity in cancer group and this increase was statistically significant (t = 649, p <0.001).

NO levels were 95.9 ± 6.1 mmol/L in healthy individuals and 139 ± 7.4 mmol/L in the breast cancer group. NO levels were also higher in the breast cancer group and the difference was statistically significant (t = 4197, P <0.001). The results were within 95% confidence intervals (CI:95 %). The results are shown in Table 1 and 2. In addition, the values are shown graphically in Chart 1. Arginase activity was done with Pearson correlation analysis found a weak correlation between the level of NO (r = 0.895, p<0.02).

Table. 1: Arginase activity and NO levels in breast cancer and control groups.

Parameters studied	Min – Max Cancer Group (n=30) (X + SE)	Min – Max Healthy Group (n=34) (X + SE)	P
Arginase Activity U/L	17.88 ±2.57 (4.0 – 76.6)	6.88 ±0.9 (0.4 – 26)	t = 3,64 p<0.01*
Levels of Nitric Oxide Micromol / L	139.4 ±7.4 (31.5 – 207.5)	95.9 ±6.1 (49.9 – 213.3)	t = 4,197 p<0.001**

*Significant for P<0.01
**Significant for P<0.001

Table. 2: Breast cancer and control groups, arginase activity and nitric oxide levels and confidence intervals.

Parameters studied	Cancer Group (n=30)	Healthy Group (n=34)
Arginase Activity confidence interval	12.6 – 23.1 (CI : %95)	.9 – 8.8 (CI : %95)
Nitric Oxide levels, confidence interval	124.1 – 154.6 (CI : %95)	83.4 – 108.3 (CI : %95)

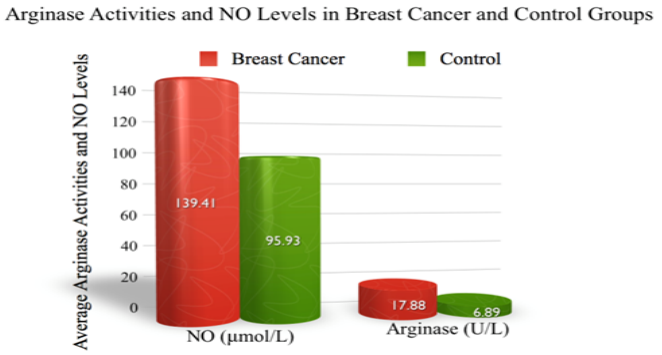

Chart. 1: Arginase activities and NO levels in breast cancer and control groups.

NO, which can easily diffuse into tissues, causes damage on cells. This molecule interacts with neuromodulators and the monoamines and stimulates their secretion into the synaptic gaps (Ohkuma and Katsura, 2001; Ceylan *et al*., 2011; Silberg *et al*., 2010). When NO, which is synthesized within the cell, diffuses out of the cell, it acts as exogenous NO and thereby stimulates the neuronal membrane depolarization (Ohkuma and Katsura, 2001). NO levels and arginase activity in our study were higher in patients with breast cancer (p<0.01). The increase in NO levels negatively affects oxidative phosphorylation and decreases peripheral oxygen intake in the patients (Shen *et al*., 1995). As a result, an energy loss occurs in brain and peripheral tissues that have not been sufficiently oxygenated. In cancer patients in whom anaerobic glycolytic pathway is preferred, as a result of all these biochemical changes, loss of energy is further increased and patients lose their life energies. Studies conducted by Savas *et al*. have also supported our results (Selek *et al*., 2008). Oxidant products and other free radicals formed by NO may have role in

the neuropathophysiology of cancer. In addition, some reseachers have also been advocated the increase in NO levels which is protective against the cyctotoxic effect of the body. This increase can also be considered as a compensatory mechanism of the body. Because NO have been demonstrated to protect cellular and extracellular structures against O, O_2, and .OH radical (Wink *et al*., 1993). After the biochemical characteristics of arginase and NOS were clearly demonstrated, the hypothesis that arginase competes with NOS for the same substrate L-arginine and thereby inhibits the synthesis of NO were proposed. Km value of NOS is 2 to 20 µmol/L, while the Km value of arginase is 1 to 5 mmol/L, but arginase activity is much higher than NOS activity at physiological concentrations of L-arginine. In this case,it has thought that both enzymes have been consumed in the same ratio (Wu and Morris, 1998; Morris, 2009). As a result, although the enzyme arginase has different Km values, it shows much more activity while using L-arginine. Genetic studies supports the idea that ariginase plays an important role in the regulation of the availability of L-arginine (Crombez and Cederbaum,2005; Iyer *et al*., 2002). Consequently, higher NO levels and arginase activity in patients with breast cancer than in controls may be biochemically associated with use of the same substrate by both NOS and arginase enzyme. As NOS isozymes function in different tissues, they may play a role depending on their mechanism of action of nNOS which is an isoenzyme in CNS. The localization of nNOS isoenzymes that is close to the subcellular calcium channels ensures that their activity is under strict control. The localization and physiology of this isoenzyme are among the subjects of current researches and the studies are currently underway (Schild *et al*., 2005). Both its physiology and the pathophysiology of the relevant diseases will be better understood with growing evidence.

Recent studies have also shown that the enzyme arginase also acts in vascular cells (Durante *et al*., 1997; Berkowitz *et al*., 2003). Although very little is known about the signaling pathways regulating the expression of arginase in these cells, cyclic adenosine monophosphate (cAMP) that is relesed in JAK(Janus Tyrosine Kinase)-STAT(Signal Transducers and Activators of Transcription) signaling pathway has been shown to induce arginase in vascular smooth muscle cells (Wei *et al*., 2000). Rho and Rho kinase also increase the activity of the endothelial arginase by thrombins (Ming *et al*., 2004; Nelin *et al*., 2005). Despite the detection of vascular arginase activity in vascular cells, the effect on vascular endothelium is not precisely known. Another study has shown that exogenous arginine is used by both iNOS and arginase, but it has not been revealed explicitly whether arginine synthesized in the urea cycle is utilized in the hepatic synthesis of NO (Stadler *et al*., 1995; Huynh and Chin-Dusting, 2006). Wu *et al* have anticipate that most of the results obtained to date may be revised and further studies will be conducted if the DNA of arginase is cloned and new information on the arginase is obtained (Wu and Morris, 1998). Another study Chang and colleagues reported that arginase activity caused by the proliferation of tumor cells. According to them, the arginase and NOS can have very different influences on the growth of nearby tumor cells depending on which pathway is prevailing (Chang *et al*., 2001).

CONCLUSIONS

Further studies are needed to determine the relationship between NO and arginase enzyme and the exact role of this results in the etiopathogenesis of cancer. In addition, in order to fully elucidate the oxidative imbalance that is thought to be implicated in the etiopathogenesis of cancer, we believe that it will be significant and appropriate to analyze NO total oxidant, as well as antioxidant parameters and even the total oxidant status and total antioxidant status. In this study, it can be concluded that there is a significant increase in arginase activity and serum levels of NO in patients with breast cancer and these parameters can be used as a marker for cancer in the diagnosis and follow-up of treatment.

COMPETİNG İNTERESTS

We have not provided financial support for working with any organization or institute.

AUTHORS' CONTRİBUTİONS

The third author (Celalettin Camci) wich data is interpreted and provided final revisions. The second author (Nurdan Ozlu Ceylan): data collection and participated in laboratory. I organized conceot/desing, statistical analyzes, drafting manuscript critical revision of manuscript approval of article, funding secured by.

ENDNOTES

[a]This study presented as an oral presentation at XXIII. National Congress of Biochemistry (December 2011, Adana / TURKEY).

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