Clinical and Experimental Health Sciences

Determination of the Plasma Levels of Growth Arrest Specific 6 in Colon Cancer Patients

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ABSTRACT

Objective: Growth arrest-specific 6 (GAS 6) has a role in cell proliferation and was detected in different cancer types. The aim of this study was to evaluate the plasma GAS 6 levels in colon cancer patients.

Methods: This was a prospective study and conducted in a research and training hospital in Turkey. Thirty-three healthy volunteers were enrolled in the control group, thirty-three colon cancer patients who were diagnosed with colon cancer for the first time. Sociodemographic characteristics of participants were recorded. Blood samples of the control group were taken once a time. Patients' blood samples were taken before and one month after surgery.

Results: There were no statistically significant differences between the sociodemographic characteristics of the two groups. The mean plasma GAS 6 levels in control were significantly higher than that of colon cancer patients ($p<.0001$). There is a statistically significant increase in GAS 6 values after surgery ($p<.0001$).

Conclusion: It was observed that plasma GAS 6 levels of the patients were lower than the control group and were elevated after surgery. We think that our study will contribute to the literature in addition to studies showing that GAS 6 levels may be associated with survival and prognosis in different cancer types.

Keywords: Colon cancer, growth arrest-specific 6, patient, plasma levels

1. INTRODUCTION

Growth arrest-specific 6 (GAS 6) is a ligand of the tyrosine kinase receptors (TKRs) (1-5). GAS 6 is a negative regulator of coagulation (1,6). It was shown that GAS 6 promotes foam cell formation in the atherosclerotic process via inducing scavenger receptor expression in vascular smooth muscle cells (1,4).

GAS 6 a vitamin K-dependent protein that was first identified in growth arrested cells (1). TKRs are important parts of intracellular signaling pathways involved in vital functions of the cell (5,6). GAS 6 has an important role in the phagocytosis of apoptotic cells in vivo (2). GAS 6 is also thought to be effective in cell proliferation because of its strong mitogenic properties for protein S smooth muscle cells and their similarity (2). GAS 6 has a role in maintaining growth and cell viability. It ensures the differentiation of cells, maintains their phagocytosis task and prevents apoptosis (1).

GAS 6 is ligand of Tyro3, Axl and Mer receptors (TAM) and acts as agonist (1). The binding affinity of GAS6 to these three receptors is Axl ≥ Tyro3 ≥ Mer, respectively (3). It is understood that GAS 6 has growth factor-like functions due to its role in intercellular adhesion and stimulating cell migration as a result of interaction with the Axl receptor (1). Axl is the most studied and has been shown to be elevated in many cancers (7). GAS 6 also has an important role in cell proliferation and phagocytosis of apoptotic cells (1-3). In vitro studies have shown that GAS 6 plays a role in cancer development and progression in various cancer cells (8). However controversial results have been reported regarding the prognostic value of the GAS 6 in different cancers (9-14). In a recent study, it was found that the GAS 6 protein has an inhibitory effect on intestinal tumorigenesis (14). According to a review, there are a few studies with GAS 6 and colorectal cancer (15). In this study, we aimed to evaluate to determine plasma GAS 6 levels in patient with colon cancer which is the third most common cancer type around the world.

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2. METHODS

This study was approved by Ethics committee of Marmara University Institute of Health Sciences Health Clinical / Human Research Ethics Committee (01.03.2013-17). This study supported by the Marmara University Scientific Research Projects Commission (project no: SAG – C – DRP – 100.713.0306). Thirty-three healthy volunteers were enrolled to the control group and thirty-three patients who first diagnosed with colon cancer were enrolled to the colon cancer patients' group. Sociodemographic characteristics of two groups were collected and recorded. Additionally, carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA) levels were recorded for colon cancer patients.

2.1. Determination of Plasma GAS 6 Levels:

Blood samples of control group were taken once a time. Colon cancer patients' blood samples were taken before and one month after surgery. Blood samples were collected from the patients for one time and kept in an Ethylenediaminetetraacetic Acid (EDTA) tubes, then centrifuged at 3500 × *g* for 15 minutes and were kept at −80 °C until the day of analysis. All blood samples were taken in the morning. We have validated Enzyme-Linked Immunosorbent Assay (ELISA) assay for the determination of GAS 6 in plasma with kits provided by R&D Systems (Minneapolis, MN, USA) as previously described in detail (16).

2.2. Statistical Analysis

Statistical Package for Social Sciences (IBM, SPSS Statistics for Windows, version 15.0. Chicago, SPSS Inc.) was used for data analysis. Data were presented as percent and mean ± standard error. Statistical significance was expressed as a *p* value <.05. Distribution of the data was assessed with Kolmogorov-Smirnov test, Shapiro-Wilk, skewness, kurtosis, and histogram. It was found that data were not normally distributed.

3. RESULTS

A total number of 33 colon cancer patients and 33 healthy volunteers were included in this study and socio-demographics features of the groups were as presented in Table 1. There were no significant differences between the groups (p>.05).

Plasma GAS 6 levels of all participants were given in Table 2. The median of the plasma GAS 6 level of control group was found to be as 10.46 ng/mL (7.12-17.13) (n=33) while it was found to be as 7.27 (3.97-10.31) ng/mL (n=33) in colon cancer patients and this difference was significant (p<.01).

Table 1. *Characteristics of the control group and colon cancer patients*

		control group (n=33)		Colon cancer patients (n=33)		p*
		n	%	n	%	
Gender	**Female**	16	48.5	13	39.4	p=.61
	Male	17	51.5%)	20	60.6	
Age (median IQR)		60 (52-69)		63 (54-72)		p=.57
Smoking status	**Yes**	4	12.1	9	27.3	p=.78
	No	29	87.9	24	72.7	
Family history of cancer	**Yes**	6	18.2	8	24.2	p=.47
	No	27	81.8	25	75.8	
Comorbid disease	**Yes**	21	63.6	22	66.7	p=.48
	No	12	36.4	11	33.3	

p: between two groups; IQR: interquartile range (25th percentile-75th percentile); Mann-Whitney U test analysis and Spearman's correlation tests were performed*

Table 2. *Plasma GAS 6 levels of control group and colon cancer patients*

	Control group (median IQR)	Colon cancer patients *(Before the surgery)* (median IQR)	p
Plasma GAS 6 levels (ng/mL)	10.46 (7.12-17.13) (n=33)	7.27 (3.97-10.31) (n=33)	p=.002

n: number of patients; IQR: interquartile range (25th percentile-75th percentile), GAS 6: growth arrest-specific 6; Mann-Whitney U test analysis was performed

The median level of CEA in colon cancer patients were found to be 31.20 (2.87-12.47) (n=22) before surgery and 1.69 (1.27-4.20) (n=22) after surgery (p<.05). Similarly, the median value of CA 19-19 was found to be 9.30 (5.28-24.00) (n=20) before surgery and 10.50 (6.35-19.95) (n=21) after surgery (p>.05) (Table 3).

Table 3. *Plasma levels of GAS 6, CEA and CA in colon cancer patients*

	Plasma GAS 6 levels (ng/mL)	Plasma CEA levels (normal range: 0-3 ng/mL)	Plasma CA 19-9 levels (normal range: 0-35 U/mL)
Before the surgery **(median, IQR)**	7.27 (3.97-10.31) (n=22)	31.20 (2.87-12.47) (n=22)	9.30 (5.28-24.00) (n=20)
One month after the surgery **(median, IQR)**	8.27 (4.75-14.63) (n=22)	1.69 (1.27-4.20) (n=22)	10.50 (6.35-19.95) (n=21)
p	p=.00002	p=.01	p=.51

n: number of patients; IQR: interquartile range (25th percentile-75th percentile), GAS6: growth arrest-specific 6; CEA: carcinoembryonic antigen; CA 19-9: carbohydrate antigen 19-9; Wilcoxon test analysis was performed

There was no significant correlation between the plasma GAS 6 and CEA levels before surgery in colon cancer (Spearman's r = 0.08; p>.05). Similarly, there was no significant correlation

between the plasma GAS 6 and CA 19-9 levels before surgery in the cancer groups (Spearman's r = 0.19; p>.05).

4. DISCUSSION

Tyrosine kinase receptors are the transmembrane proteins, forming extracellular signals to provide adhesion and motility of cells so help the cell living, growth, differentiation (17). In the pathogenesis of many forms of cancer, the increase observed in the secretion or activities of TKR become a current issue and this will help for the investigation of new therapies (18,19). In our study have two results. The first result presents the determination of the plasma GAS 6 levels in colon cancer patients. The second result presents the evaluation of the GAS 6 levels before and after surgery.

In a previous study it has been shown that GAS 6 levels increased in thyroid cancer (10). In another study, GAS 6 over expression is mostly observed in acute myeloid leukemia (AML) and it was reported that GAS 6 expressed by AML blasts could be a marker of poor clinical outcomes (20). Similarly, it was found that high expression of activated Axl was an independent predictor for worse prognosis in patients with osteosarcoma (21). In contrast it was shown that increased activation of tissue GAS 6 levels in the kidney was associated with good prognosis in patients with renal cell carcinoma (22). In another previous study it was shown that higher expression of GAS 6 in breast cancer tissue was associated with improved outcomes (11). Similarly, it was found that a positive correlation was observed between increased GAS6 levels in the tissues of patients with brain tumors and worsening of prognosis (23).

Uribe et al. reported that Axl promotes migration and invasion in colorectal cancer (24). In contrast to this study, Akitake – Kawano et al. showed that higher GAS 6 plasma levels were associated with better survival in patients with colorectal cancer (14). Similarly, we found that the mean plasma GAS 6 levels of patients with colon cancer, were significantly lower than those of the control group (p<.05). In this study, plasma GAS 6 levels increased one month after surgery in patients with colon cancer (p<.05).

Limitations: The number of patients in our study, which was conducted within the scope of the doctoral thesis, is small due to time constraints. In addition, the fact that the Axl levels of the patients could not be evaluated is another limitation.

5. CONCLUSION

In conclusion, considering the results of this study in colon cancer patients, changes in GAS 6 plasma levels were found to be correlated positively with the changes in colorectal cancer markers. In addition, depending on our results and the literature (15,24,25), the increase in GAS 6 plasma levels after surgery suggests that GAS 6 may be used to monitor and evaluate the success of treatment. However further randomized controlled studies are needed.

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Research idea: ST, FVI, FU
Design of the study: ST, FU, FVI, OBO
Acquisition of data for the study: ST, FVI, FU, OBO, VA, PFY
Analysis of data for the study: ST, OBO
Interpretation of data for the study: ST
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