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Changes in PDGF concentration in surgically treated colorectal carcinoma

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ABSTRACT

Purpose: The aim of the present study was to asses the effect of tumor advancement, differentiation grade and surgery treatment on PDGF- AB level and platelet (PLT) count depending on the site of blood collection.

Material and methods: The study included 38 patients submitted to surgical treatment due to diagnosis of colorectal cancer (CRC) without remote metastasis: G2- 20 patients and group G3- 18 patients. The control group consisted of 24 healthy subjects. In CRC patients the blood samples was collected three times: 1) before surgery, 2) intrasurgically and 3) 90 days after surgery. Serum PDGF- AB concentration was determined by ELISA- Kit reagents.

Results: PDGF concentration in all the patients was several times higher than in the control group, irrespective of tumor differentiation grade and the site of blood collection. However the level of PDGF- AB in intraoperatively collected arterial blood and venous blood in group G3 (arterial blood- 379.9±12.1ng/ml; venous blood- 398.4±13.2 ng/ml) was significantly higher than in group G2 (arterial blood- 169.4 ±88.6 ng/ml; venous blood- 194.2±84.0 ng/ml). No significant differences were observed between venous and arterial blood. No correlation was found between the PLT count and PDGF- AB concentration.

Conclusion: High blood PDGF- AB concentration in CRC patients but no significant positive correlation observed between the PLT count and PDGF- AB suggest its neoplastic origin beside PLT. Determination of this factor in blood serum may have an important implication in early diagnosis of CRC, which is the second most common malignant neoplasm with high recurrence rates.

Key words: colorectal carcinoma (CRC), PDGF- AB (platelet- derived growth factor- AB), platelets (PLT)

INTRODUCTION

The platelet- derived growth factor (PDGF), a micromolecular protein secreted from numerous cells, is found in cells and blood serum but not in plasma [1]. It was first identified and described as a mitogenic factor secreted from the alpha granules of platelets during blood coagulation [2,3]. Subsequent studies have shown its presence in vascular endothelial cells, fibroblasts, smooth muscle cells and in great amounts in tumor tissue [4-7]. Histopathological studies have revealed that tumor cells and fibroblasts in the tumor stroma resistant to PDGF action increase the autocrine production of this factor [8-9].

PDGF is not a single molecule, but constitutes a group of proteins that build up 4 types of chains, i.e. PDGF-A, PDGF-B, PDGF-C and PDGF-D, which in the active form occur as dimers. The PDGF-A and PDGF-B chains may give rise to 3 isoforms that form homodimers AA and BB or heterodimer AB, differently expressed in various cell types. The homodimers CC and DD originate from PDGF-C and PDGF-D chains [8,10-12].

The biological role of PDGF involves the regulation of cell growth and differentiation, and is associated with its chemotactic properties toward neutrophils and monocytes. PDGF participates in angiogenesis and neoangiogenesis, and in wound healing it stimulates the synthesis of collagen I and III and glycosaminoglycans [1].

Table 1. **PDGF (ng/ml) levels in colorectal adenocarcinoma patients; before - before resection of colorectal adenocarcinoma, after 3 months after surgery, in intraoperatively collected arterial (aa) and venous (vv) blood, and in the control group.**

Time	PDGF concentration (ng/ml)			
of collected blood	**Group (G2) (N = 20)**	**Group (G3) (N = 18)**	**Control group (C) (N = 24)**	**p**
	x ± SD	x ± SD	x ± SD	
Before surgery	208.1 ± 82.4	379.0 ± 121.0	34.3 ± 9.2	G2 vs G3 p * ; G2 and G3 vs C p***
Post surgery	78.9 ± 30.4	244.3 ± 69.8	34.3 ± 9.2	G2 vs G3 p** ; G2 and G3 vs C p***
Intrasurgically (aa)	169.3 ± 88.5	379.0 ± 92.0		G2 vs G3 p**
Intrasurgically (vv)	194.2 ± 84.0	398.4 ± 101.9		G2 vs G3 p**

Group G2 - ($T_{2-3}N_{1-2}M_0$ histopathological type G2), Group G3 - ($T_{2-3}N_{1-2}M_0$ histopathological type G3), *p<0.05; ** p<0.01; *** p<0.001.

PDGF exerts its effect on cells through two structurally related receptors α and β [13,14]. Fibroblasts and smooth muscle cells possess the two receptors, blood platelets have only one receptor α and macrophages only one receptor β [15]. The receptors are tyrosine kinases showing different affinity for PDGF. The receptor α (170 kDa) binds all PDGF isoforms while the receptor β (180kDa) binds only isoforms containing the PDGF-B chain. Interesting is the fact that different cells show different expression of these two receptors, depending on their activation and proliferation. For instance, the number of β receptors on connective tissue cells is small, but increases during the inflammatory process and neoplastic transformation [8,16].

Colorectal carcinoma (CRC) is one of the most common malignancies in Poland, with the second highest incidence irrespective of age. The prevalence rate increases with age, particularly over the age of 50. The risk of recurrence of the so called metachronic adenocarcinoma (even after 10 years) refers to approximately 4% of patients considered surgically cured [1]. As the progression of benign lesions to malignant tumors is a long process (approximately 10 years) there is a constant search for noninvasive diagnostic methods that would facilitate monitoring of its dynamics. This would allow implementation of pharmacological therapy to delay neoplastic progression whenever surgical treatment cannot be applied. According to literature survey, PDGF is an early marker of colorectal carcinoma growth. As reported by some researchers, an increased PDGF level stimulates both proliferation and dissemination of tumor cells [12].

The lack of unequivocal data evaluating blood serum PDGF levels before and after surgery inspired us to undertake further investigations in this field in relation to differentiation malignancy grade.

The aim of the present study was to assess the surgery treatment and the effect of tumor advancement, malignancy grade on the PDGF level and platelet count depending on the site of blood collection.

MATERIALS AND METHODS

The protocol was approved by the Medical University of Bialystok Bioethics Commitete, according to Guidelines for Good Clinical Practice (GCP) (R-I-003/325/2004). Only patients who gave their informed written consent were enrolled in the study.

The study included 38 colorectal cancer patients (CRC) without remote metastasis (20 men and 18 women; aged 42 - 68 years) diagnosed and operated in the Department of General Surgery at the J. Sniadecki Hospital in Bialystok and 24 healthy subjects (control group) (10 men and 14 women; aged 40 - 64 years).

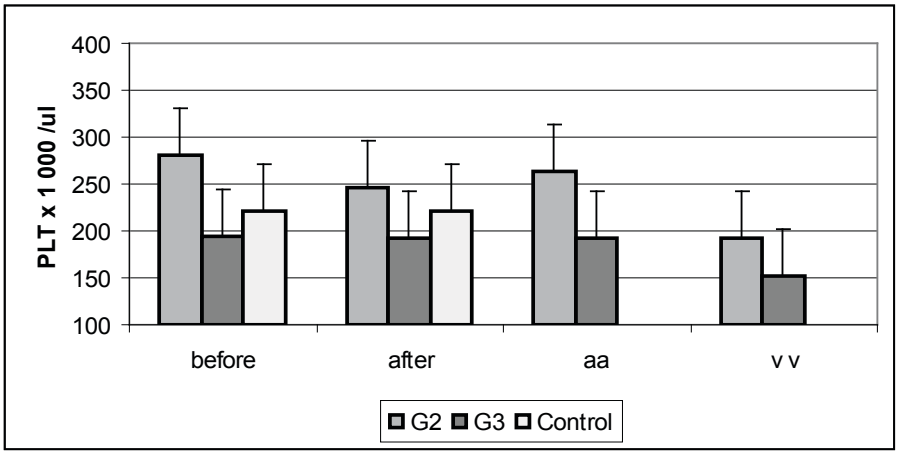
The diagnosis of colorectal cancer was based on clinical symptoms, abdominal ultrasound scanning, and colonoscopy. The patients undergone surgical procedure of tumor resection with subsequent regional lymphadenectomy. During surgery, samples (primary tumor, lymph node involvement) were collected and neoplastic lesions were histopathologically verified. All patients had CRC. None of the patients received chemotherapy or radiation therapy before and post surgical intervention.

The colorectal cancer patients qualified for our study did not have any other ailment that could affect the immune response. Those patients who had a recent inflammatory or any acute infection were excluded from this study. Clinical advancement was evaluated based on TNM classification by Hutter and Sobin [17], while tumor type and malignancy grade were determined by histopathological analysis.

CRS patients were divided into two groups: Group G2 – 20 patients ($T_{2-3}N_{1-2}M_0$, malignancy differentiation grade G2) and Group G3 – 18 patients ($T_{2-3}N_{1-2}M_0$, malignancy differentiation grade G3). The control group consisted of 24 healthy subjects.

Blood collected in clot tubes was the material used for determination of PDGF-AB and in EDTA-K2 tubes – to assess the PLT count. Blood samples were collected three times: 1) prior to surgery (peripheral blood), 2) intrasurgically (from the afferent and efferent vessels within the region of tumor mass) and 3) 3 months after surgery (peripheral blood from the ulnar vein).

Figure 1. **PLT count in colorectal adenocarcinoma patients before resection of colorectal adenocarcinoma (before), 3 months after surgery (after), in intraoperatively collected arterial (aa) and venous (vv) blood, and in the control group.**



Group G2 ($T_{2-3}N_{1-2}M_0$ histopathological type G 2), Group G3 ($T_{2-3}N_{1-2}M_0$ histopathological type G 3), Group C – control.

PDGF-AB was measured in blood serum using commercially available immunoenzymatic method with the human PDGF-AB ELISA-Kit (Bender MedSystem, Viena, Austria), while PLT count - using a hematological analyzer SYSMEX.

Statistical analysis

Nonparametric U Mann-Whitney test and Spearman correlation test were used for statistical analysis of the results. The program STATISTICA 6.0 for Windows was applied. Differences were considered statistically significant for $p<0.05$.

RESULTS

In all the study patients, the PDGF-AB concentration in peripheral venous blood was several times higher than in the control group, both before and after surgery ($p<0.001$) (*Tab. 1*). However, statistically significantly higher values were found in group G3: before treatment (379.0±121.0 ng/ml), after treatment (244.4±69.8 ng/ml) ($p<0.05$) as compared to group G2: before treatment (208.1±82.4 ng/ml), after treatment (78.9±30.4 ng/ml) ($p<0.01$) (*Tab. 1*). According to the Spearmann's coefficient, group G2 showed a positive correlation between PDGF level before and after surgery ($r = 0.78$ $p<0.05$). No such correlation was found in group G3 level before and after surgery ($r = 0.14$ $p > 0.05$).

A comparison of PDGF values between venous and arterial blood collected during surgery from the region of tumor mass revealed no statistically significant differences ($p<0.05$) (*Tab. 1*) However, the PDGF level in group G3 was found to be approximately twice as high as in group G2, both in venous and arterial blood (*Tab. 1*), (G2 vs G3 $p<0.01$) (*Tab. 1*). According to Spearmann's coefficient, the analysis showed a positive correlation between PDFG in arterial and venous blood collected intrasurgically in both study group G2 ($r = 0.60$ $p = 0.024$) and G3 ($r = 0.74$ $p = 0.035$).

The PLT count was higher before and after surgery when compared to the control group (*Fig. 1*). The analysis of PLT count in venous and arterial blood collected during surgery from the region of the tumor mass showed higher PLT count in arterial blood in both groups ($p<0.001$). However, the PLT count was found to be lower in group G3 as compared to group G2 (*Fig. 1*). In the analysis of Spearmann's correlation coefficients the PDGF level did not correlate with PLT count prior to surgery and intrasurgically. However, 3 months after tumor resection, both PDGF concentration and PLT count became reduced, although they were still significantly elevated but no showed positive correlation ($r = 0.38$ $p>0.05$).

DISCUSSION

As neoplastic progression is known to be accompanied by thromboembolic disturbances associated with enhanced activation, adhesion and platelet aggregation, we determined the PLT count, which in all our study patients was statistically significantly higher before than after surgery, irrespective of tumor malignancy grade.

Our studies shown that the PLT count was higher before and after surgery compared to the control group. The analysis of PLT count in venous and arterial blood collected during surgery from the region of the tumor mass showed higher PLT count in arterial blood in both groups. However, the PLT count was found to be lower in group G3 as compared to group G2. Colorectal carcinoma frequently predisposes to thromboembolic complications, which are the second cause of mortality in these patients. Blood platelets actively participate in the pathogenesis of thrombosis promoted by the inflammatory process. Inflammation is characterized by mutual interactions between PLT, leukocytes and endothelial cells, with a significant role of active platelet surface receptor CD62P [18]. Platelet activation is accompanied by a release of many

proangiogenic factors and mediators that trigger modulate the immune response neoplastic cells and stimulate growth, i.e. PDGF [19]. Moreover, platelets interact with neoplastic cells facilitate formation of metastases and participate in microvascularization of tumors.

The platelet-derived growth factor (PDGF) was first identified and described as a mitogenic factor secreted from the alpha granules of platelets during blood coagulation [2]. Subsequent studies have shown its presence in vascular endothelial cells, fibroblasts, smooth muscle cells and in great amounts in tumor tissue [7]. Histopathological studies have revealed that tumor cells and fibroblasts in the tumor stroma resistant to PDGF action increase the autocrine production of this factor [9]. The PDGF is an important factor that regulates migration and proliferation of a number of cells. As a mitogenic factor, it exerts an effect on cells through specific surface receptors. PDGF does not occur in plasma and is released to blood serum during thromboembolic disorders that constantly occur in the neoplastic tissue due to the dense vasculature and an abundant source of cancer procoagulant (CP) [11].

The results obtained in our study revealed a significantly increased concentration of PDGF in colorectal cancer patients as compared to the healthy population, which confirms platelet activation in cancer patients. The finding of a statistically significant correlation between level and histopatological type is interesting. We obtained higher concentration of PDFG in patients with higher histopatological differentiation G_3 (379.0 ng/ml), as compared to lower differentiation G_2 (169.3ng/ul). The correlation between PDFG and differentiation type may indicate that the increase in PDFG level as a marker of platelet activation *in vivo* is associated with the presence of tumor. The positive correlation observed between the PLT count and PDGF concentration shown its neoplastic origin beside PLT.

We found higher, statistically significant PDGF level in all patients operated on for colorectal carcinoma as compared to healthy controls, irrespective of tumor malignancy grade and the site of blood collection. We also noted similar levels of PDGF in peripheral venous blood and the blood collected intraoperatively. However, the level was found to increase with tumor malignancy, thus suggesting a phenotypical strong expression of PDGF from neoplastic cells to blood. Therefore, the assessment of serum PDGF level allows investigation of malignant conversion dynamics. Worthy of note is also the fact that teh peripheral venous blood is a biological material that is much easier to obtain than tumor biopsy or sections.

No statistically significant differences were found in the PDGF level between peripheral venous blood and venous and arterial blood collected intraoperatively from the region of tumor mass in both group G2 and G3. However, we found higher, statistically significant PDGF level in patients with higher differentiation malignancy grade (Group G3). The similar level of PDGF in blood serum, irrespective of the site of blood collection, may indicate that neoplastic tissue is the major source of PDGF. Other sources may include: PLT, smooth muscle cells of the vascular wall, vascular endothelial cells, as well as monocytes and macrophages constituting leukocytes infiltration [1,5]. The few times higher PDGF level in patients operated on for colorectal carcinoma as compared to the control group may suggest a slow and successive release of PDGF from cells to blood. Moreover, the higher PDGF level and the lower PLT count in patients with higher differentiation malignancy grade suggest that the neoplastic tissue and also PLT are the major PDGF sources. However, no positive correlation was found PLT count and PDGF level in the studies groups.

Neoplastic formation is a multi-stage process, controlled by many genes. Tumor progression is also due to the expression of adhesive molecules, also PDGF, which as a mitogen stimulates cell proliferation through G_0-G_1 cell cycle phase shift [5]. PDGF is a pleotrophic family of peptide growth factors that signal through cell surface, tyrosine kinase receptors (PDGFR α and β) and stimulate various cellular functions including growth, proliferation, and differentiation. To date, PDGF expression has been demonstrated in a variety of cancers, including colorectal cancer [12]. Colorectal carcinoma is the second most common cause of cancer deaths. However, the role of PDGF and PDGF-R in therapeutic strategies of colon carcinoma has not been reported yet. Our studies shown that the stimulation of neoplastic progression is associated with loss of cell adhesion in perineoplastic tissue caused by increased PDGF expression and due to enhanced migration of granulocytes and macrophages, which eventually leads to leukocytes infiltration with an inflammatory reaction in perineoplastic tissue.

Cancer procoagulant released from neoplastic tissue increases blood prothrombotic condition which usually accompanies malignant neoplasms, including colorectal carcinoma. The state of increased coagulability stimulates thrombopoiesis, which has been confirmed by our findings showing a higher, statistically significant platelet count before than after surgery. This fact can be explained by normalization of thrombopoiesis after tumor resection.

We have shown an important effect of tumor differentiation malignancy grade on the level of PDGF in blood serum. This factor released from neoplastic cells is a chemotactic and mitogenic factor for mesenchymal cells. It can be thus assumed that PDGF plays an essential role in neoplastic metastases, in correlation with differentiation malignancy grade.

There is an evidence that in neoplastic tissue PDGF is secreted as an autocrine and paracrine factor [2], which may confirm the drop in PDGF level after surgical resection of neoplastic lesions in our study. We observed an elevated PDGF level in patients with higher differentiation malignancy grade (G3) of colorectal cancer, which can be another explanation of the hypothesis that neoplastic tissue is an autocrine source of PDGF.

The inhibition of PDGF activity and other cytokines through blockage of specific receptors suggests future therapeutic strategy. Determination of this factor in blood serum may improve early diagnosis of colorectal carcinoma

and allow treatment monitoring. Since this CRC is known to give frequent recurrences, the assessment of PDGF level can serve as a pivotal index of the disease relapse. The investigation of PDGF change dynamics may play an important role in the evaluation of malignant conversion.

PDGF concentration was higher in all CRC patients irrespective of differentiation malignancy grade and site of blood collection as compared to the control group. Prior to CRC resection, PDGF concentration was higher than after surgery, and still significantly higher as compared to healthy subjects. The sources of PDGF serum level were not only PLT but also tumor tissue.

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