CONCENTRATION OF METALLOPROTEINASE-2 AND TISSUE INHIBITOR OF METALLOPROTEINASE-2 IN THE SERUM OF PATIENTS WITH BENIGN AND MALIGNANT THYROID TUMOURS TREATED SURGICALLY

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Objectives. Neoplastic angiogenesis is an essential stage of growth, progression and invasion of solid tumours. The process of basement membrane degradation and remodelling of the extracellular matrix (EMC) involves proteolytic enzymes called metalloproteinases. Among the numerous proteolytic enzymes of this group the key role is played by metalloproteinase-2 (MMP-2) and tissue inhibitor of metalloproteinase 2 (TIMP-2). Tissue expression and concentration of these compounds in body fluids have been used in early diagnostics of tumours development, assessment of tumours advancement and treatment results monitoring. The aim of the study was to evaluate the concentration of MMP-2 and TIMP-2 in blood serum of patients with benign and malignant thyroid tumours and the effect of surgical treatment on these parameters in the postoperative period as well as assessment whether to MMP-2 and TIMP-2 serum concentration in patients with thyroid cancer positively correlates with the clinical staging classification of the International Union Against Cancer (UICC).

Patients and methods. The study group consisted of 53 patients with various types of thyroid cancer and 23 patients with benign thyroid tumours, while 26 healthy adults served as controls. According to clinical staging classification of thyroid cancer the 32 patients were classified with stage I, 6 with stage II, 8 with stage III and 7 with stage IV. We have found higher mean concentration of MMP-2 in 53 patients with thyroid cancer as compared to the control group and the group of 23 patients with benign thyroid tumours. All patients were treated operatively. Additionally, a significant effect of radical surgical treatment on mean concentration of MMP-2 and TIMP-2 in patients with papillary and follicular thyroid cancer was demonstrated.

Conclusions. MMP-2 and its tissue inhibitor TIMP-2 apparently play a significant role in the pathogenesis of thyroid cancer. Evaluation of their concentration in peripheral blood serum may be useful for the differentiation between benign and malignant thyroid tumours. Serum MMP-2 and TIMP-2 concentrations in patients with thyroid cancer did not significantly correlate with the clinical staging of thyroid cancer.

Key words: metalloproteinase 2 - tissue inhibitor of metalloproteinase 2 - thyroid cancer - thyreoidectomy - clinical staging of cancer

Formation of new blood vessels in the process of angiogenesis is connected with proliferation and migration of endothelial cells. Endothelial cells first invade the surrounding extracellular matrix (ECM). As observed by INGBERG and FOLKMAN (1989) the process involves numerous interrelated interactions, and many proangiogenic factors. Main mediators of this process include: growth factors, proteins of extracellular matrix, cellular adhesive molecules and numerous proteolytic enzymes. Neoplastic angiogenesis, the process

of formation of blood vessels within solid tumours, is an essential stage of growth, progression and invasion of these neoplasms. This complex process is initiated by damage to the basement membrane of a vessel and remodelling of the extracellular matrix, which enables migration of endothelial cells towards the source of angiogenic signal, i.e. neoplastic cells. The process of basement membrane degradation and ECM remodelling involves proteolytic enzymes called metalloproteinases (MMPs). The first metalloproteinase extracted by Gross and Lapiere (1962) was interstitial collagenase. Docherty et al. (1985) identified further seven metalloproteinases and Stetler-Stevenson et al. (1989) identified two tissue inhibitors of these metalloproteinases (TIMP). Presently, over 20 endopeptidases have been described, showing activity against the majority of macromolecules of the extracellular matrix and their 4 tissue inhibitors. Kohn et al. (1994) found that metalloproteinases are endopeptidases connected with a zinc atom, active in neutral pH and in the presence of calcium ions. Depending on the structure, localisation within the cell and type of substrate, metalloproteinases can be divided into 5 main groups: collagenases, gelatinases, stromyelisins, matrylisins and membrane metalloproteinases (MT-MMPs). MMPs are released as an inactive proenzyme (zymogene), in which catalytic zinc ion is bond to the cysteine rest. The majority of proMMPs are activated by plasmin, formed from inactive plasminogen by urokinase plasminogen activator (uPA). Natural inhibitors of MMPs are tissue inhibitors of metalloproteinases (TIMPs). There are four types of TIMPs (TIMP-1,-2,-3,-4). TIMPs inhibit the activity of MMPs forming non-covalent complexes resistant to proteolysis. Lack of balance between the proteolytic activity of MMPs and the activity of their inhibitors (TIMPs) is one of the elements necessary for the growth and progression of solid tumours as observed by Talbot et al. (1996). Hanemaaijer et al. (1993) found that endothelial cells are able to produce some MMPs, including: MMP-1,-2,-3,-9. Hofmann A et al. (1988) observed that most enzymes of the MT-MMP/MMP class of proteases facilitating invasion of thyroid tumor cells seem to have been produced by fibroblasts, not by the tumor cells them selves. Among these metalloproteinases, the constitutional one showing the greatest expression in endothelial cells is MMP-2, also called gelatinase A. TIMP-2 is its natural tissue inhibitor, at the same time necessary for the activation of MMP-2.

Important role played by MMP-2 and TIMP-2 in the pathogenesis of solid tumours led to attempts of applying the evaluation of their tissue expression and concentration in body fluids in early diagnostics of tumours, assessment of tumours advancement and treatment results monitoring.

The aim of this study was to evaluate the concentration of MMP-2 and TIMP-2 in blood serum of patients with benign and malignant thyroid tumours, the effect of surgical treatment on these parameters in the postoperative period and to answer the question whether concentration of the above mentioned factors in serum correlate with the clinical staging of thyroid cancer. We used the clinical staging of thyroid cancer, introduced by the International Union Against Cancer (UICC) in 2002. Apart from such parameters as tumor size (T), regional lymph node status (N) and the presence of distant metastases (M), this classification also takes patient' s age into consideration. Criteria, worked out by the International Union Against Cancer, permit a precise assessment of the clinical staging of thyroid cancer.

Patients and Methods

Patients. The study comprised patients operated on at the Clinic of General and Endocrinological Surgery, Institute of Endocrinology, Medical University of Lodz for benign and malignant thyroid tumours. The first group comprised 53 patients (40 F, 13 M), mean age 45.96±11.5 years, in whom preoperative cytological study of fine needle aspiration biopsy specimens led to the diagnosis of thyroid cancer. The material obtained during operation, subjected to routine histopathologic examination, was used for the verification of the diagnosis. The final diagnosis was as follows: 36 cases of papillary thyroid cancer (PTC), 7 follicular thyroid cancers (FTC), 5 medullary thyroid cancers (MTC) and 5 cases of anaplastic thyroid cancer (ATC). Intraoperative evaluation and postoperative histopathology was the basis for division of all studied thyroid cancers according to the clinical staging of thyroid cancer, introduced by the International Union Against Cancer (UICC). According to the clinical staging, we qualified patients with thyroid cancer as stages I (32 patients), II (6 patients), III (8 patients) and IV (7 patients). The second group consisted of 23 patients (18 females, 8 males), mean age 40.19±19.3 years with diagnosed simple nodular goiter (SNN). Cytological evaluation of material from thin needle aspiration biopsy revealed the presence of follicular nodule *neoplasma folliculare* (NF). In postoperative histopathologic examination the diagnosis was verified as benign tumour – follicular adenoma. The control group comprised 26

healthy volunteers (20 females, 6 males), mean age 41.03±13.7 years, in whom clinical examination, evaluation of thyroid hormones concentration (fT_3; fT_4), concentration of TSH and ultrasound examination excluded thyroid diseases.

Treatment. The studied patients were operatively treated. In cases of differentiated thyroid cancer complete struma resection was done with modified lymphadenectomy (removal of central cervical lymph nodes, lateral cervical lymph nodes without jugular vein, sternocleidomastoid muscle and nerve XI). The completeness of operative treatment was based on postoperative ultrasound, iodine uptake in cervical scintigraphy after stimulation with endogenous TSH and histopathologic examination. In patients with anaplastic tumours, due to the advancement of the process, the operation was limited to reduction of the tumour mass. In patients with simple nodular goiter a lobectomy and subtotal resection of other lobe was performed.

The concentration of MMP-2 and TIMP-2 was measured in blood serum sampled in aseptic conditions from a peripheral vein on the day preceding the operation, and 4 weeks after the operation. The study was approved of by the Ethical Committee of the Medical University of Lodz. Determinations of MMP-2 were done using commercial kits of ELISA test from Amersham Biosciences (inter-assay CV – 5.6 %; intra-assay CV – 10 %), and TIMP2 from Amersham Pharmacia Biotech (inter-assay CV – 3.9 %; intra-assay CV – 4.8 %). The results were presented as mean values ± mean standard error (SEM), and statistical analysis was done between the measured parameters using Student's t-test. The values were statistically significant at $p<0.05$. We examined a correlation between the staging of thyroid cancer and mean values of investigated factor concentrations. Pearson correlation coefficient r was determined and regression line was plotted.

Results

We have found statistically significantly higher concentrations of MMP-2 in blood serum of patients with thyroid cancer as compared with the control group (1346.79±22.11 vs. 1231.73±59.85 ng/ml; $p<0.05$). Within particular thyroid cancer groups higher mean concentrations of MMP-2 were also seen when compared with the control group. Statistical significance was noted in the papillary thyroid cancer (PTC) group (1363.19±27.58 vs. 1231.73±59.85; ng/mL $p<0.05$). In patients with benign tumours the concentration of MMP-2 was significantly lower than in the control group (1071.73±41.49 vs. 1231.73±59.85 ng/mL; $p<0.05$) (Figure 1). Mean concentration of TIMP-2 in blood serum of thyroid cancer patients was higher than in the control group, but the difference was not significant (155.75±24.55 vs. 115.62±6.28 ng/mL; $p>0.05$). In particular cancer types mean TIMP was higher than in the control group, but this difference also was not significant. In patients with benign thyroid tumours the concentration of TIMP2 was lower than that in the control group, but with no statistical significance (Figure 2).

When compared between thyroid cancer group and that with benign tumours, mean concentration of MMP-2 was significantly higher in thyroid cancer patients (e.g. 1346.79±22.11 vs. 1071.73±41.49 ng/mL; $p<0.001$). In particular thyroid cancer types mean concentration of MMP-2 was significantly higher than that in the group with SNN(NF): in the PTC group (1363.19±27.58 vs. 1071.73±41.49 ng/mL; $p<0.001$), follicular thyroid cancer (FTC) group (1281.43±67.57 vs. 1071.73±41.49 ng/mL; $p<0.05$), ATC group (1320.00±44.02 vs. 1071.73±41.49 ng/mL; $p<0.05$), medullary thyroid cancer (MTC) group (1347.00±75.87 vs. 1071.73±41.49 ng/mL; $p<0.05$) (Figure 3). Mean blood serum concentration of TIMP-2 was higher in the thyroid cancer group than that in benign tumours, but the difference was not significant statistically (155.75±24.55 vs. 105.02±18.50 ng/mL; $p>0.05$). In particular types of cancer mean concentration of TIMP-2 was higher than in the SNN(NF) group, but the differences were also not significant statistically (Figure 4). We compared the blood serum concentration of MMP-2 and TIMP2 before and after the operation. After radical thyreoidectomy the level of MMP-2 decreased. Statistically significant decrease of this parameter was noted after thyreoidectomy for PTC (1363.19±27.58 vs. 1128.19±45.51 ng/mL; $p<0.001$); FTC (1281.43±67.57 vs. 1096.43±66.38 ng/mL; $p<0.005$) (Figure 5). Mean concentration of TIMP2 after thyreoidectomy significantly decreased in patients with PTC (169.74±35.97 vs. 94.25±9.53 ng/mL; $p<0.05$) and FTC (121.66±10.72 vs. 104.74±11.63 ng/mL; $p<0.05$). In the MTC group, however, it did not change significantly after the operation (Figure 6). After palliative procedure for ATC an increase of both studied parameters in the postoperative period was observed, but only the concentration of MMP-2 was statistically significantly higher after the operation than before (1320.00±44.02 vs. 1609.00±57.69 ng/mL; $p<0.05$) (Figure 5). After partial struma resection in SNN(NF) a significant increase of MMP-2 was noted in the postoperative period

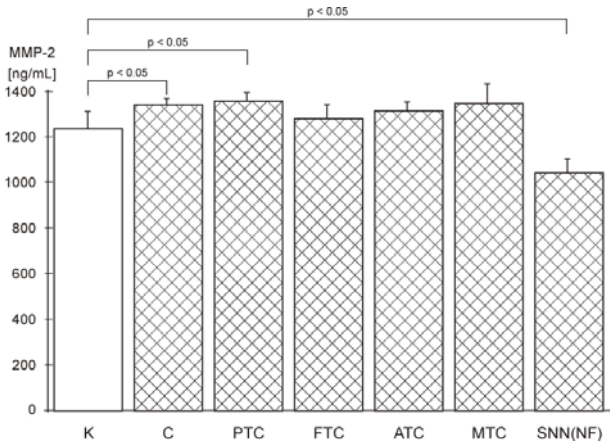

Fig 1 Mean concentration of MMP2 ± SEM in the group of patients with thyroid cancer (C) and its particular types (PTC – papillary thyroid cancer, FTC – follicular thyroid cancer, ATC – anaplastic thyroid cancer, MTC – medullary thyroid cancer) and in the group of patients with benign thyroid tumours (SNN NF) vs. control group (K)

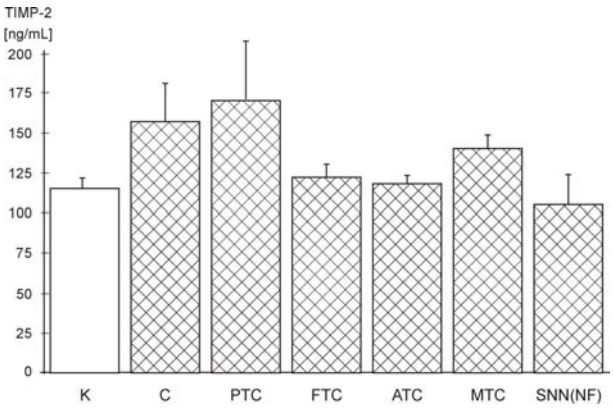

Fig 2 Mean concentration of TIMP2 ± SEM in the group of patients with thyroid cancer (C) and its particular types (for legend see Fig. 1), and in the group of patients with benign thyroid tumours (SNN NF) vs. control group (K).

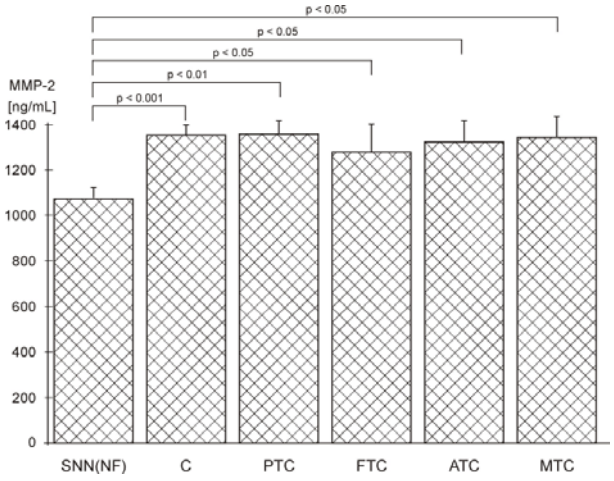

Fig 3 Mean concentration of MMP2 ± SEM in the group of patients with thyroid cancer (C) and its particular types (for legend see Fig. 1) vs. patients with benign thyroid tumours (SNN NF)

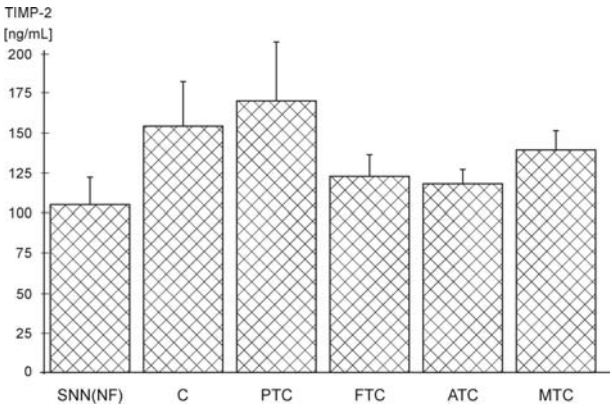

Fig 4 Mean concentration of TIMP2 ± SEM in the group of patients with thyroid cancer (C) and its particular types (for legend see Fig. 1) vs. patients with benign thyroid tumours (SNN NF)

(1071.73±41.49 vs. 1203.85±57.21 ng/mL; p<0.01) (Figure 5) and statistically insignificant decrease of the concentration of TIMP-2 (Figure 6).

Table 1 presents mean examined MMP-2 and TIMP-2 concentrations +/- standard error of mean (SEM) of the respective stages of thyroid cancer.

We investigated if correlation existed between mean analyzed neoangiogenesis mediator concentrations and the staging of thyroid cancer. To achieve this objective, we determined Pearson correlation coefficient r and regression line was plotted. However, no significanmt correlation has been found between the staging of thyroid cancer and mean MMP-2 concentration (y=12.22x + 1324.7; r=0.0856; p>0.05) and between the staging of thyroid cancer and mean VEGF concentration (y = -8.0227x + 170.27 r=-0.0506, p>0.05).

Discussion

For a long time the biological function of MMPs was thought to be limited to their participation in the decomposition and degradation of extracellular matrix components and basal membrane in various physiological and pathological processes. The concept that

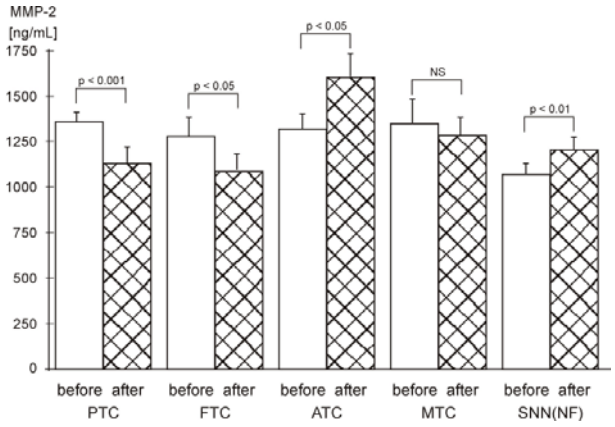

Fig 5 Mean concentration of MMP2± SEM in the group of patients with various types of thyroid cancer (for legend see Fig. 1), and in the group of patients with benign thyroid tumours (SNN NF) before and after surgery. NS – statistically not significant

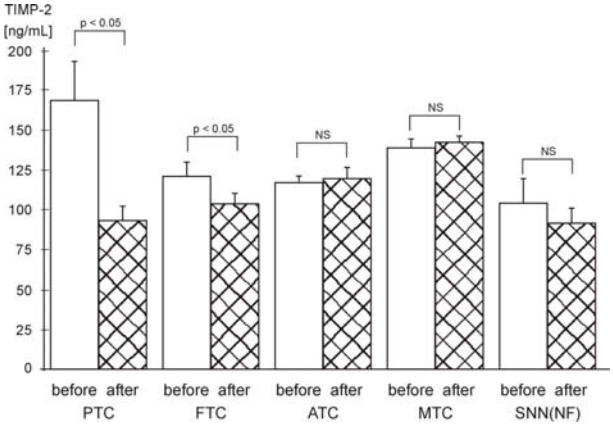

Fig 6 Mean concentration of TIMP2± SEM in the group of patients with various types of thyroid cancer (for legend see Fig. 1) and in the group of patients with benign thyroid tumours (SNN NF) before and after surgery. NS – statistically not significant

Table 1.
Mean MMP-2 and TIMP-2 concentrations ± standard error of mean (SEM) of the respective stages of thyroid cancer

stages of cancer	MMP-2 [ng/ml]	TIMP-2 [ng/ml]
I	1327.7±33,6	148.4±27.7
II	1410.0±45,8	280.1±161.4
III	1380.6±35.5	123.8±9.8
IV	1341.4±33.8	119.3±2.1

MMPs activity in the process of neogenesis is limited only to the invasion of basal membrane and destruction of ECM elements in distant metastases has been modified and nowadays various effects of this group of proteolytic enzymes in tumour progression are stressed. MMPs play a key role in tumour development and growth, in neoangiogenesis, penetration and migration of cancer cells into and out of blood vessels and colonisation of distant tissues. Kraiem et al. (2000) found that the role of tissue inhibitors of metalloproteinases (TIMP) is not limited to anti-invasive properties and inhibition of MMPs, but they also participate in the activation of MMP and tumour growth. Among metalloproteinases constitutively produced by endothelial cells the highest expression is demonstrated by MMP-2. Therefore, evaluation of its concentration in body fluids and tissue expression of this enzyme and its tissue inhibitor TIMP2 are thought to have a potential in the assessment of advancement of solid tumours. Numerous researchers have used various techniques to confirm the prognostic value of evaluation of the increase of tissue expression of MMP-2 and lack of balance between MMP-2 and TIMP-2 in various forms of solid tumours. Several authors (Mori et al. 1997; Theret et al. 1997; Talvensaari-Mattila et al. 1998; Gong et al. 2000; Silletti and Cheresh 1999; Yamamura et al. 2002; Young and al. 1966) observed a significant correlation between the increase in MMP-2 expression and poor prognosis in stomach cancer, colon cancer, breast, pancreas, prostate, urinary bladder, lung and ovarian cancer. Numerous authors reported increased MMP concentration in such body fluids as: serum, peripheral blood plasma and urine. Zucker et al. (1999) reported high concentration of MMP in blood plasma in patients with colonic, breast, prostate and urinary bladder cancer. Several authors (Sasaki et al. 2002a; Sasaki et al. 2002b; Korem et al. 2002) reported significantly increased concentration of MMP-2 in blood serum of patients with lung cancer, thymoma and adrenal cancer. There are numerous reports on the inhibitory effect of TIMP2 on the growth of solid tumours and distant metastasing. Bramhall et al. (1996) confirm close correlation between the reduction of tissue expression of TIMP-2 and increase in the aggressiveness of pancreatic cancer. Several authors (Murashige et al. 1996; Ree et al. 1997; Fong et al. 1996) observed positive correlation between an increase of TIMP expression and poor prognosis of colonic cancer, breast and lung cancer. There are few reports on the application of metalloproteinases (and their tissue inhibitors) levels evaluation in the assessment of endocrine glands tumours advancement and monitoring treatment results. The

aim of the study was evaluation of the concentration of MMP-2 and TIMP-2 in blood serum of patients with benign and malignant thyroid tumours and the effect of surgical treatment on these parameters in the postoperative period. We found increased mean concentration of MMP-2 in all types of thyroid cancer, as compared with the control group. However, statistical significance was present only in papillary thyroid cancer. This fact is due to higher number of patients in this group than in other thyroid cancer types.

It is also noteworthy that mean concentration of MMP-2 and TIMP-2 in blood serum of patients with thyroid cancer was higher than in benign thyroid tumours group. However, the differences were statistically significant only for MMP-2. This fact justifies the application of the assessment of MMP-2 and TIMP-2 concentration in differentiation between benign and malignant thyroid tumours. There was no significant correlation between the clinical staging of thyroid cancer and mean MMP-2 and TIMP-2 concentration.

Our results confirm significant role of MMP-2 and TIMP-2 in the process of thyroid cancer development, their usefulness in the diagnostics and differentiation of benign and malignant thyroid tumours. The results are consistent with Maeta et al. (2001) observation on increased tissue expression of MMP-2 and TIMP-2 in thyroid cancer. Korem et al. (2002) demonstrated the diagnostic value of tissue MMP-2 expression evaluation but lack of its prognostic value. This is consistent with our findings on the usefulness of MMP-2 serum concentration evaluation in differentiation between benign and malignant thyroid tumours. The fact of significant decrease of mean MMP-2 and TIMP-2 concentration after radical surgery confirms reports on their constitutive expression in thyroid cancer cells. Evaluation of MMP-2 blood serum concentration in long postoperative period may become an additional parameter in the monitoring of operative treatment radicality and early detection of recurrences.

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